METABOLOMICS SOCIETY

15th Annual Conference of the Metabolomics Society

METABOLOMICS 2019

JUNE 23–27 | THE HAGUE

ORAL AND POSTER ABSTRACTS

AGENDA AT A GLANCE

Biomedical | Plant, Food, Environmental and Microbial | Technology | New Frontiers

SUNDAY, JUNE 23

	Atlantic	Alexia	Ariane	Oceania Foyer
11:00 a.m.	REGISTRATION OPEN			
12:30 p.m. – 2:15 p.m.	**W1:** EMN – Data Fusion	**W2:** Mining the Metabolome	**W3:** Multi-Omics Integration & Systems Metabolomics	**Intro to the Field 1:** Data Acquisition
2:30 p.m. – 4:15 p.m.	**W4:** Application of Graphical Models to Metabolomics	**W5:** Plant Metabolomes: Natural & Generated Variability	**W6:** How to Link Metabolome & Genome Mining	**Intro to the Field 1:** Data Acquisition
4:30 p.m. – 6:15 p.m.	**W7:** EMN – Professional Career Development	**W8:** Putting Metabolomic Data into Context	**W9:** An Open-Source Pipeline for Appraisal of NMR Datasets	**Intro to the Field 1:** Data Acquisition
6:30 p.m. – 8:30 p.m.	**Career Night** – Pacific			

MONDAY, JUNE 24

	Atlantic	Alexia	Ariane	Oceania Foyer
7:30 a.m.	REGISTRATION / INFO DESK OPEN			
8:30 a.m. – 10:15 a.m.	**W10:** The Importance of Quality Assurance & Quality Control	**W11:** Towards FAIR Spectral Libraries	**W12:** EMN – Stable Isotope-Resolved Metabolomics	**Intro to the Field 2:** Data (pre) Processing and Biostatistics
10:30 a.m. – 12:15 p.m.	**W13:** Beyond pathway mapping	**W14:** Standardizing the Fluxomics Workflows	**W15:** Tools to Study the Microbiome-Metabolome Interplay	**Intro to the Field 2:** Data (pre) Processing and Biostatistics
12:15 p.m. – 1:30 p.m.	LUNCH BREAK – ON YOUR OWN			
1:30 p.m. – 3:15 p.m.	**W16:** Application Metabolomics in Industry	**W17:** Dynamic Modeling of Human Metabolism	**W18:** EMN – Volatomics in Human Health	**Intro to the Field 2:** Data (pre) Processing and Biostatistics
3:30 p.m. – 5:00 p.m.	**Opening Ceremony** | **Plenary Session 1** – Joshua Rabinowitz – King Willem-Alexander Hall			
5:15 p.m. – 6:45 p.m.	**Welcome Reception – Poster Session 1** – Odd Numbers – Exhibit Foyer			
7:00 p.m. – 8:00 p.m.	**Metabolomics Society Town Hall Meeting –** Atlantic			

TUESDAY, JUNE 25

	Atlantic	King Willem-Alexander	Alexia	Ariane
7:45 a.m.	REGISTRATION / INFO DESK OPEN			
8:30 a.m. – 9:30 a.m.	**Plenary Session 2** – Dorret Boomsma – King Willem-Alexander Hall			
9:30 a.m. – 10:15 a.m.	BREAK – EXHIBIT FOYER			
10:15 a.m. – 12:00 p.m.	**1.** Cancer	**2.** Plant Applications 1	**3.** Data Integration & Data Basing 1	**4.** Novel Technologies
12:00 p.m. – 1:30 p.m.	LUNCH – IN FOYER WITH EXHIBITS – PLATINUM SPONSOR PRESENTATIONS			
12:20 p.m. – 1:20 p.m.			**Sponsor Pres:** SCIEX	**Sponsor Pres:** Waters Corporation
1:30 p.m. – 3:15 p.m.	**5.** Metabolic Disease	**6.** Food Applications 1	**7.** Flux Studies	**8.** Novel Instruments, Tools and Services
3:15 p.m. – 3:45 p.m.	BREAK – EXHIBIT FOYER			
3:45 p.m. – 5:30 p.m.	**9.** Epidemiology	**10.** Microbial Applications	**11.** Metabolite Identification 1	**12.** Stem Cells, Organoids
5:30 p.m. – 7:00 p.m.	**Poster Session 2** – Odd Numbers – Exhibit Foyer			
7:00 p.m. – 8:30 p.m.	**EMN Reception** – Pacific			

WEDNESDAY, JUNE 26

	Atlantic	King Willem-Alexander	Alexia	Ariane
8:00 a.m.	REGISTRATION / INFO DESK OPEN			
8:30 a.m. – 9:30 a.m.	**Plenary Session 3** – Cathie Martin – King Willem-Alexander Hall			
9:30 a.m. – 10:15 a.m.	BREAK – EXHIBIT FOYER			
10:15 a.m. – 12:00 p.m.	**13.** Lipidomics and Cardiovascular Disease	**14.** Plant Defense	**15.** Data Analysis & Statistics	**16.** Single Cell
12:00 p.m. – 1:30 p.m.	LUNCH – IN FOYER WITH EXHIBITS – PLATINUM SPONSOR PRESENTATIONS			
12:20 p.m. – 1:20 p.m.			**Sponsor Pres:** Thermo Fisher Scientific	**Sponsor Pres:** Shimadzu Europa GmbH
1:30 p.m. – 3:15 p.m.	**17.** Ageing and Disease	**18.** Food Applications 2	**19.** Data Integration & Data Basing 2	**20.** Regulatory Session
3:15 p.m. – 3:45 p.m.	BREAK – EXHIBIT FOYER			
3:45 p.m. – 5:30 p.m.	**21.** Infection and Immunity	**22.** Environment & Toxicology	**23.** New Instrumentation	**24.** Novel Applications
5:30 p.m. – 7:00 p.m.	**Poster Session 3** – Even Numbers – Exhibit Foyer			
7:30 p.m. – 10:30 p.m.	**Conference Dinner – Xiringuito**			

THURSDAY, JUNE 27

	Atlantic	King Willem-Alexander	Alexia	Ariane
8:30 a.m.	REGISTRATION / INFO DESK OPEN			
8:45 a.m. – 10:00 a.m.	**25.** Respiratory Diseases		**26.** Model Organisms	**27.** Genome-scale Modeling
10:00 a.m. – 10:45 a.m.	**Poster Session 4** – Even Numbers – Exhibit Foyer			
10:45 a.m. – 12:00 p.m.	**28.** Microbiome		**29.** Plant Applications 2	**30.** Metabolite Identification 2
12:00 p.m. – 1:30 p.m.	LUNCH – IN FOYER WITH EXHIBITS – PLATINUM SPONSOR PRESENTATIONS			
12:20 p.m. – 1:20 p.m			**Sponsor Pres:** Bruker Daltonics	**Sponsor Pres:** Agilent Technologies
1:30 p.m. – 3:30 p.m	**Plenary Session 4** – Jean-Charles Portais – **Closing Ceremony** – King Willem-Alexander Hall			

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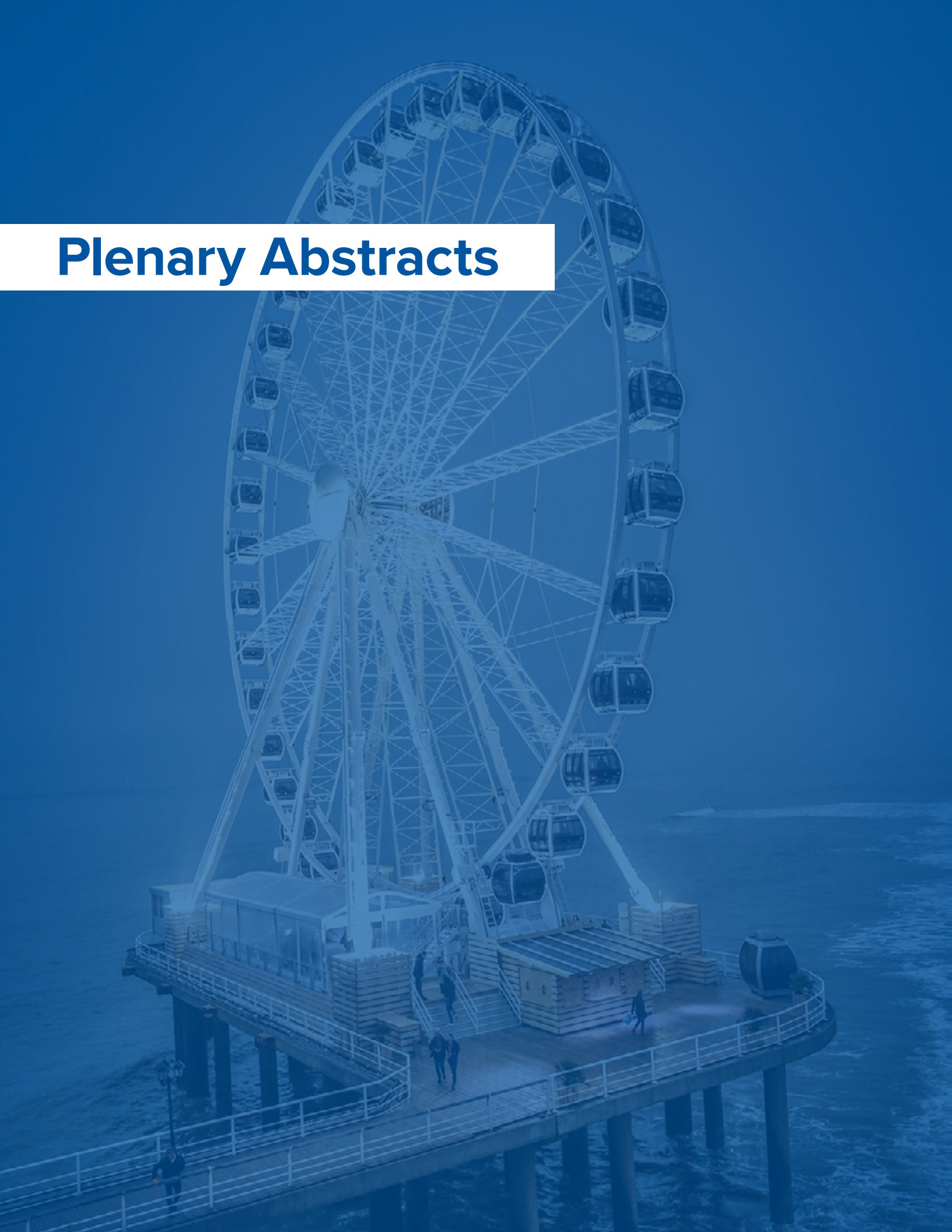
CONTENTS

METABOLOMICS SOCIETY

Plenary Abstracts



PLENARY SESSION 1

The marriage of technology development and biological discovery

Monday, June 24, 4:00 p.m. – 5:00 p.m.
PRESENTING AUTHOR: Joshua Rabinowitz, Lewis-Sigler Institute, United States

Today, 20 years after sequencing of the first human chromosome, we now have powerful tools for measuring all of the fundamental classes of biomolecules, including metabolites. While the data gathering capacity of these tools is inspiring, the complexity of biology remains humbling. The failure of these tools to substantially eliminate major diseases, including metabolic disease, is self-evident. In this lecture, I will describe our past and ongoing efforts to bridge metabolomic technology development and biological discovery. I will provide examples of how intensive biological focus improved our metabolomic pipelines, and of how isotope tracing illuminated otherwise obscure aspects of metabolism. Examples will be drawn from microbiology, cell biology, and cancer. A major focus will be on our recent efforts to revisit quantitatively mammalian metabolism, with an eye towards better understanding of both basic metabolic principles and the intersection of diet, metabolism, and disease.

PLENARY SESSION 2

Twin and SNP-based studies of metabolomics traits

Tuesday, June 25, 8:30 a.m. – 9:30 a.m.
PRESENTING AUTHOR: Dorret I. Boomsma, Netherlands Twin Register, Vrije Universiteit, Netherlands

Metabolite profiling allows for an increased understanding of behavioral and psychiatric phenotypes. We hypothesized that promising metabolites in this respect include those for which a genetic component is present estimated heritability (h2: standardized estimate of genetic variance) based on the different genetic relatedness of mono- and dizygotic twin pairs.

Metabolomics data from 4 different platforms for up to 5,117 individuals, including some family members of twins. We estimated that ~50% of variation is due to genetic variation, but heritability estimates differed across metabolite classes and lipid species.

Heritability also can be estimated from genotype data (SNPs: Single Nucleotide Polymorphisms) by quantifying the genetic similarity of 'unrelated' individuals and comparing it to their trait similarity (h2SNP). To elucidate the genomic architecture of blood metabolites, we simultaneously estimated total heritability (h2total), SNP heritability (h2SNP) and the proportion of heritability captured by known metabolite loci (h2GW-loci). We reviewed all association studies of SNPs and blood metabolites, retrieving 241,965 associations for which all associated metabolites were classified. For the estimation of h2GW-loci we aggregated > 800 class-specific metabolite loci for 309 lipids and 52 organic acids. This revealed significant differences in h2SNP and h2GW-loci among different classes of lipids and organic acids. Phosphatidylcholines with a higher degree of unsaturation had higher h2GW-loci estimates.

This study highlights the genetic architecture of metabolite classes and lipid species, with genes explaining on average 50% of variation in metabolites, and common genetic variants influencing metabolites. This knowledge can be used in construction of polygenic scores and causal modeling.

PLENARY SESSION 3

The future for food may be in our past: the importance of plants in our diets.

Wednesday, June 26, 8:30 a.m. – 9:30 a.m.
PRESENTING AUTHOR: Cathie Martin, John Innes Centre, Colney Research Park, UK

The appreciation of the challenges of achieving global food security has matured to include nutritional security, as scientists have realised that not only calorie content but food and colonic microbial composition impact our health and well-being, dramatically. The ways that the nutrients we consume affect our health are highly complex due to the diversity of what we eat, the varying digestibility of what we eat, the changing composition and functioning of each individual's gut microbiota, the differences in absorption and bioavailability of the nutrients we eat, the differences in responses between individuals to what they eat and the multi-fold mechanisms of action that nutrients have on our health. It has been accepted for more than 50 years that diets rich in plants, particularly fruit and vegetables, protect health, and yet diets have declined, with lower fruit and vegetable content replaced by more cheap, sugary, oily processed foods. These dietary shifts have had a marked impact on the incidence of chronic diseases; obesity, metabolic diseases, type2 diabetes and cardiovascular diseases.

Only by understanding how phytonutrients improve our health by reducing risks of chronic disease and which phytonutrients confer greatest benefits in protecting against specific diseases (a process termed comparative nutrition) can we hope to achieve dietary improvements at all levels in society. By describing examples of preclinical comparative nutritional analyses, I hope to illustrate the potential of dietary improvement using plant-based foods to improve our health and quality of life and to reduce the economic burden on our health-care systems.

PLENARY SESSION 4

Comprehensive investigations of cancer cell metabolism using 13C-fluxomics

Thursday, June 27, 1:30 p.m. – 2:30 p.m.
PRESENTING AUTHOR: Jean-Charles Portais, University Toulouse, France

Metabolism is a basic cellular function that sustains survival, growth and adaptation of living organisms. At the cellular level, metabolism is organized as a network, i.e. a complex set of biochemical reactions that are tightly interconnected. To investigate such complex systems, powerful tools are required, and a broad range of approaches (metabolomics, fluxomics) have been developed over the past decade to provide detailed and quantitative measurement of the operation of metabolic networks. These methods can be combined with other omics tools (e.g. transcriptomics, proteomics, etc.) and to metabolic modelling to provide systems biology approaches for in-depth, comprehensive understanding of the organization, functioning and adaptation of cellular metabolism. This understanding can provide the rationale for efficient and cost-effective optimization of production systems (knowledge-based metabolic optimization) in biotechnology, or to characterize the metabolic dysfunctions associated with pathologies, or to identify and validate new therapeutic targets or strategies. In this lecture, particular emphasis will be made on 13C-fluxomics, with selected illustrations in the field of cancer metabolism, and how this can help in designing improved therapeutical strategies.

VENDOR SESSION

SESSION 8: NOVEL INSTRUMENTATIONS, TOOLS AND SERVICES *Presented by Platinum and Gold Sponsors*

Tuesday, June 25 | 1:30 p.m. – 3:15 p.m.
Princess Ariane

By participating in this session you will get an overview of the latest developments in instrumentation and tools (from our 6 platinum sponsors) and services (from our 4 gold sponsors). Each company will highlight their latest developments in a 7-minute flash presentation. The session is scheduled at the start of the conference, allowing you to determine which exhibitors will provide the most valuable follow-up visit at their booth during the event. The interactive session also has 2 panel discussions, moderated by Thomas Hankemeier and David Wishart.

1:30 p.m. – 2:30 p.m.	PLATINUM PRESENTERS AND PANEL DISCUSSION

Agilent Technologies
Christine Miller, Omics Market Manager, USA

A New LC/Q-TOF Platform for Metabolomics Analysis
Innovations in LC/MS and GC/MS have driven the field of metabolomics. Speed, sensitivity, resolution, dynamic range, and isotopic fidelity are all important MS attributes for metabolomics research. This presentation will describe some of the innovative technologies that are in the new Agilent 6546 LC/Q-TOF platform which is designed with metabolomics research in mind.

Bruker Daltonics/Bruker BioSpin
Lucy Woods, phD, Product Manager QTOF, Germany

New ground-breaking releases to advance 4D Metabolomics and Lipidomics
Get to know Brukers´ latest innovations for 4D metabolomics and lipidomics research. We cordially invite you learn more about our newest developments for SpatialOMx and NMR-based metabolic fingerprinting. Be ready to get surprised!

SCIEX
Baljit Ubhi, Market Manager - Metabolomics Business, USA

Technology Innovations for Advancing Metabolomics at SCIEX
Technology advancements will be presented highlighting SCIEX's innovation in improving healthcare and wellness. Analytical hardware and software tools for metabolomics and lipidomics highlight their utility for enabling precision medicine.

Shimadzu
Emily Armitage, Research Scientist, UK

Driving data-driven decision making just a little bit quicker...
For many metabolomics workflows, there is a need for a smarter path to data-driven decision-making and to help find actionable data. This headline overview will highlight the way in which high resolution accurate mass with data independent acquisition methods can make a difference to metabolomic workflows. The talk will focus on the Shimadzu LCMS-9030 QTOF platform and software tools to help accelerate data-driven decision making in metabolomics using DIA and a research application to find components and help verify identification.

Thermo Fisher Scientific
Amanda Souza, Manager, Product Marketing, USA

7 Minutes of mzCloud Spectral Library: We'll Leave You Begging for More
Unknown annotation is one of the most difficult challenges for untargeted metabolomics researchers. Learn how the mzCloud spectral library accelerates the unknown annotation process using more spectral information across diverse chemical classes to ultimately annotate more with greater confidence.

Waters Corporation
David Heywood, Senior Manager Omics Business Development, United Kingdom

From out of the box methods to structural characterization. Solutions for metabolomics research
To fully understand the complex relationship between biology and the compounds we measure, multiple approaches and workflows need to be considered. Discovery workflows provide indications of metabolic perturbation often needing supplemental measurements across large cohorts, measurement of metabolic flux or even clarification of compound structure. This presentation will discuss how Waters innovates with purpose to address the challenges in Metabolomics research.

VENDOR SESSION CONTINUED

SESSION 8: NOVEL INSTRUMENTATIONS, TOOLS AND SERVICES *Presented by Platinum and Gold Sponsors*

Tuesday, June 25 | 1:30 p.m. – 3:15 p.m.
Princess Ariane

By participating in this session you will get an overview of the latest developments in instrumentation and tools (from our 6 platinum sponsors) and services (from our 4 gold sponsors). Each company will highlight their latest developments in a 7-minute flash presentation. The session is scheduled at the start of the conference, allowing you to determine which exhibitors will provide the most valuable follow-up visit at their booth during the event. The interactive session also has 2 panel discussions, moderated by Thomas Hankemeier and David Wishart.

2:30 p.m. – 3:15 p.m.	GOLD PRESENTERS AND PANEL DISCUSSION

Biocrates Life Sciences AG

Therese Koal, PhD, Head of Research & Development, Austria

Unlock Nutrition-Microbiome-Host Interaction with Metabolomics

Intestinal bacteria influence a vast range of biological processes and have been associated with the pathogenesis and the course of many diseases. However, establishing associations is not sufficient to understand the functional interaction between nutrition, microbiota and the host organism. As metabolic processes are a key element of this interaction, metabolomics has evolved as an important tool for functional microbiomics studies.

This talk will be discussing the relevance of selected metabolic pathways in this context. It will also present the new MxP® Quant 500 Kit as an analytical tool to assess a variety of microbiota-derived metabolites, including bile acids, indoles and TMAO. The MxP® Quant 500 Kit is the most comprehensive solution within Biocrates' portfolio of kits for Targeted Metabolic Profiling, covering up to 630 compounds from 26 metabolite and lipid classes. It enables researchers to obtain detailed information about endogenous metabolism as well as the systemic impact of microbial activity.

Cambridge Isotope Laboratories, Inc.

Krista Backiel, Marketing Manager and Metabolomics Manager, USA

Stable Isotope-Labeled Mixes for Multiplexed Metabolomics: Production and Application

One of the aims of metabolomics research is to quantitatively measure metabolites in biosamples. The impetus is to better understand disease mechanisms and to identify or validate candidate biomarkers toward improved personalized medicine. The field has made significant progress over the past decade in addressing these aims. Efforts of which have been aided by advancements in analytical methodology, technology, software, as well as reagents (e.g., stable isotope-labeled standards). To assist in the high throughput and multiplexed analysis of metabolites, we have formulated (and are in the process of formulating) an array of isotope-enriched, multi-component mixtures. This presentation will chronicle the process of producing multi-component mixtures, from standard preparation at our production facility through to the assembly of the isotope-enriched mix at our chemical formulations laboratory. Additionally to be discussed is the application of our isotopically labeled mix in targeted or untargeted MS metabolomic studies.

Human Metabolome Technologies

Tom Hoshiba, Managing Director, Netherlands

HMT Europe

Japanese biotech company Human Metabolome Technologies (HMT) opened an European Office in Leiden, Netherlands.

Metabolon

Alex Forrest-Hay, VP Population Health, USA

Delivering Actionable Insights with Metabolomics

There are 4 CORE capabilities essential to insight-deriving metabolomics.

REGULATORY SESSION

SESSION 20: REGULATORY SESSION

Translating metabolomics from academic science into regulatory practice: challenges and progress in pharmacology and toxicology

Wednesday, June 26 | 1:30 p.m. – 3:15 p.m.

While metabolomics has become a mature and widely used technology in academic research, its application to regulatory science has been limited to date. Several factors contribute to this slow uptake, the most commonly cited roadblocks are the lack of standardisation, validation and reporting formats for metabolomics. Recently, progress has been made in translating 'omics technologies (including metabolomics) from academic science towards regulatory practice in the fields of pharmacology and toxicology, specifically for drug and chemical safety, respectively. This timely session at Metabolomics-2019 will introduce and review some of the opportunities, challenges and recent progress in this translational activity. While focused on drug and chemical safety legislation in Europe, the progress reported will have much wider implications for metabolomics in international regulatory practice. Talks will be presented by regulators, industry and academic scientists to provide a balanced perspective, and include a panel discussion chaired by Dr. Pim Leonards (Vrije Universiteit Amsterdam).

An Overview of the 21st Century Cures Act: Opportunities for Biomarkers and Precision Medicine

Dr. Rick Beger, National Center for Toxicological Research, US FDA, United States

A biomarker is defined as a characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure, or therapeutic intervention. There are seven BEST biomarker categories; diagnostic, monitoring, pharmacodynamic response, predictive, prognostic, safety, and susceptibility/risk. The 21st Century Cures Act establishes a biomarker "qualification" process for drug development tools that can then be used for particular context of use (COU) in a regulatory submission. The submission of a biomarker for regulatory use under the 21st Century Cures Act, is a three-stage submission process and builds on the existing biomarker submission process. The first Stage is a letter of intent (LOI), that includes the drug development need the biomarker is intended to address, biomarker information. The intended context of use (COU), and information on how the biomarker will be measured. If the FDA accepts the LOI, a qualification plan (QP) describing the information including the analytical method and performance characteristics that will qualify the biomarker for the proposed COU in drug development is submitted. The third step is a full qualification package (FQP) that contains all accumulated information on the biomarker, organized by topic area. The FDA will make a final decision about whether the biomarker is qualified based on the FQP. The 21st Century Cures Act and metabolomics ability to capture phenotype information on a patient provides opportunities and for biomarkers and precision medicine. Examples of potential metabolomics biomarkers will be provided.

Big Data "Omics" – Challenges and Opportunities in Regulation

Dr. Renate König, Paul-Ehrlich-Institut, Federal Institute for Vaccines and Biomedicines, Germany

A new Joint HMA/EMA Task Force was established, initiated by EU Heads of Medicines Agencies (HMA) in collaboration with the European Medicines Agency (EMA), to explore how medicines regulators can use Big Data to support research, innovation and robust medicines development to benefit human health.

This talk will focus on the identification of the emerging challenges from the use of Big Data, with the focus on Bioanalytical Omics, to prepare the European regulatory system for the future, on establishing recommendations for use of "Omics" big data in assessment of medicines as well highlight the opportunities for "Omics" in the regulation of medicines.

Various Applications of Metabolomics in Toxicology

Prof Hennicke Kamp, BASF, Germany

BASF and its daughter company metanomics established the database MetaMap®Tox containing the plasma metabolome of more than 1000 compounds derived from 28-day studies in rats. Approximately 250 different endogenous metabolites are measured and more than 100 patterns for different toxicological modes of action are available. MetaMap®Tox is nowadays routinely used at BASF to support systemic toxicity testing in terms of identification of target organs and modes of action or for read-across applications (Kamp at al., 2012; van Ravenzwaay et al., 2016). Over the last years, we have broadened this approach to identify maternal toxicity in reproductive toxicity studies (Keller et al., 2018). Additionally, a highly stable and reproducible liver in vitro model was established, in which the intracellular metabolome of HepG2 cells can be specifically altered through treatment with different hepatotoxicants (Ramirez et al., 2017). So far more than 90 different treatments have been analysed with this setup. In this presentation, examples will be shown how metabolomics can be used for the purposes mentioned above. Since the acceptance of such approaches in the regulatory community is somehow limited, also concepts for increasing regulatory acceptance will be discussed.

SESSION 20: REGULATORY SESSION

Translating metabolomics from academic science into regulatory practice: challenges and progress in pharmacology and toxicology

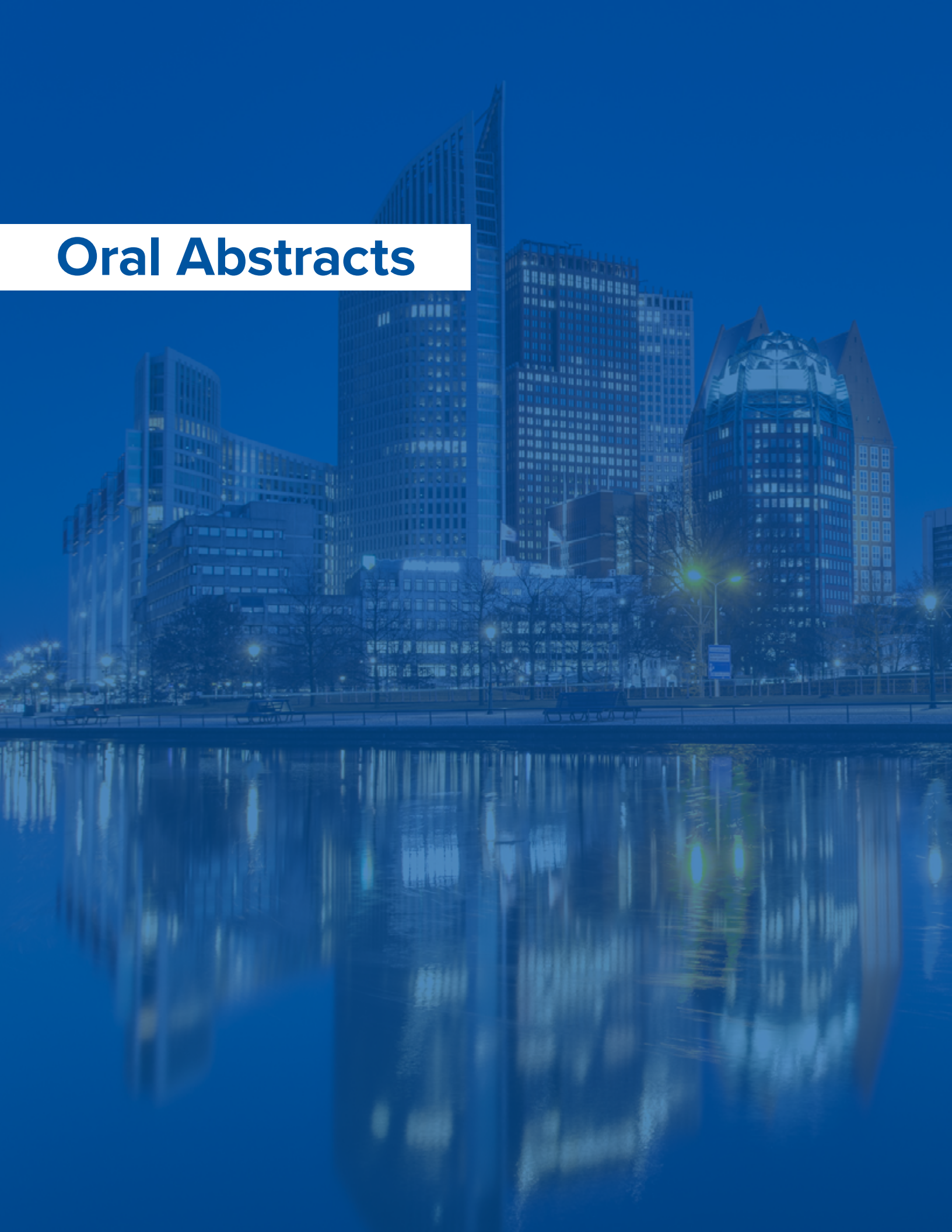
Wednesday, June 26 | 1:30 p.m. – 3:15 p.m.

Best Practice Guidelines and Reporting Standards for Applications of Metabolomics in Regulatory Toxicology: the International MERIT Project

Prof. Mark Viant, University of Birmingham, UK

During the last two decades metabolomics has become a very widely used technology in academic research, yet its application to regulatory science, including regulatory toxicology, has been limited. While several factors contribute to this slow translation into regulatory practice, the most commonly cited roadblock by chemical regulators, government and industry scientists is the lack of standardisation, best practice guidelines and data and metadata reporting formats. Here we will describe the final report of the MEtabolomics standaRds Initiative in Toxicology (MERIT) project, which brought together a team of international experts from multiple sectors to address this need. MERIT sought to define best practice guidelines and minimal reporting standards for designing, undertaking, reporting and assessing the quality of both untargeted metabolomics and targeted metabolite analyses in the context of regulatory toxicology. To provide context, the most relevant use cases for metabolomics in toxicology were also identified, including for example chemical grouping based upon metabolic phenotypes. The MERIT guidelines included experimental design; QA/QC; sampling and extraction; data acquisition and processing of mass spectrometry and NMR spectroscopy assays; data processing and statistical analysis; and metabolite identification. A strategy and architecture for integrated data management, sharing and exploitation in regulatory toxicology was also developed. We concluded that most steps in the application of untargeted metabolomics and targeted metabolite assays to regulatory toxicology are well established, including significant recent progress in QA/QC. We recommend that the MERIT minimal reporting standards and data management strategy are tested as multi-stakeholder use cases.

Oral Abstracts



BIOMEDICAL

SESSION 1: CANCER	Tuesday, June 25	10:15 a.m. – 12:00 p.m.

1A Session Keynote 10:15 a.m. – 10:45 a.m.	Metabolomics Applications for Oncology and Immunology in Drug Discovery

PRESENTING AUTHOR: ***Thomas Roddy, Agios Pharmaceuticals, United States***

Metabolomics data from in vitro and in vivo studies is more comprehensive and in depth than ever. Large data sets from metabolomics and flux studies are often daunting to analyze and interpret, especially when projects move quickly. A major focus of our lab has been in developing workflows and data analytics to allow rapid turnaround of data and fast iteration of biological experiments. Our goal is to quickly learn from one experiment, to design the next, and to iterate this process in a way that effectively impacts our projects, allowing us to identify novel biological mechanisms and pharmacodynamic biomarkers for our oncology and rare genetic disease programs. In this presentation, I will briefly discuss our multidisciplinary MS lab (metabolomics, lipidomics, fluxomics, and proteomics) and data systems. I will also present examples when metabolomics gave us unique insights into biological processes, including the development of a pharmacodynamic marker for a cholesterol pathway oncology target, an analysis of the tumor microenvironment across several models, and the modulation of metabolism in immunological models (in vitro and in vivo).

1B 10:45 a.m. – 11:05 a.m.	Multi-omic discovery of metabolic rewiring in triple-negative breast cancer following mitochondrial folate transport ablation

PRESENTING AUTHOR: ***Steven Gross, Weill Cornell Medicine, United States***

CO-AUTHORS: ***Qiuying Chen, Joshua B. Zuk, Chris A. Miller, Steve M. Fischer, Steven S. Gross***

Mitochondrial tetrahydrofolate (THF)-mediated reactions provide one-carbon units for synthesis of purines, thymidine, and methylation of DNA, RNA, proteins, and lipids. Inhibition of THF-mediated reactions (i.e., antifolates) have been a staple of cancer chemotherapy, including triple-negative breast cancer (TNBC). The present study sought to broadly discover how TNBC cells retaliate to knockout of the mitochondrial THF transporter (MFT/slc25a32), and consequent loss of mitochondria-dependent one-carbon trafficking reactions. Using untargeted metabolomics, discovery proteomics and untargeted stable isotope tracing strategy, we discover that mitochondria folate transporter (slc25a32) gene deletion in TNBC cells elicits a profound rewiring of glucose to polyol pathway, concomitant with delayed cell cycle and a 6-10-fold increase in several aldo-keto reductases (AKRs) - enzymes that consume NADPH to facilitate cytosolic one carbon reaction that generates NADPH. Activation of polyol pathway and switch of serine-derived one carbon metabolism from mitochondria to cytosol were similarly observed in methotrexate treated TNBC cells. Our findings nominate these upregulated AKR enzymes as chemotherapeutic targets, for inhibition in combination with antifolate drugs. Additionally, multi-omics studies revealed that loss of slc25a32 increases TNBC cell dependence on purine salvage, and TGF-β signaling with evidence of epithelial-mesenchymal-transition (i.e., increased cell migration/invasion). Dependence of upregulated polyol pathway, purine salvage, cytosolic one carbon reaction and TGF-β signaling was supported by downregulation in slc25a32 protein-restored KO cells. Taken together, multi-omic findings provide unexpected insights into how TNBC cells adapt to disabled mitochondrial folate-dependent one-carbon metabolism, and suggest novel therapeutic targets for combined intervention with antifolate drugs.

1C 11:05 a.m. – 11:20 a.m.	Investigating the metabolic response of cancer cells to indisulam

PRESENTING AUTHOR: ***Lili Herendi, PhD student, United Kingdom***

CO-AUTHORS: ***Anke Nijhuis, Arti Sikka, Eirini Koulura, Orli Yogev, Louis Chesler, Gerald Larrouy-Maumus, Hector Keun***

Neuroblastoma (NB) is one of the most common type of solid tumour in infants. Currently there are no reliable biomarkers identified predicting disease outcome in patients within the high-risk group highlighting the acute need for novel therapies. Indisulam (E7070) an aryl sulphonamide, a carbonic anhydrase IX (CAIX) inhibitor currently in Phase II clinical trials for the treatment of patients with solid tumours has recently been connected with pre-mRNA splicing factor RNA binding protein 39 (RBM39). Recruitment of RBM39 by indisulam to the DCAF15/CUL4/E3 complex promotes proteasomal degradation. These findings connect cancer cell line sensitivity to indisulam with stability of RBM39 and high expression of DCAF15. Our data confirmed sensitivity of IMR32 human NB cells to indisulam both in vitro and in vivo. In IMR32, the DCAF15 dependent RBM39 degradation and mis-splicing were necessary, but not sufficient for the full cytotoxic response to indisulam. Using LC-MS and GC-MS metabolomics analysis together with stable isotope labelling we have identified the activity of indisulam to be associated with alterations in metabolite pools connected with NADH production and changes in redox balance. Furthermore, as mitochondrial respiration was also impaired after indisulam treatment, these metabolic alterations observed imply that neither CAIX nor RBM39 are the sole targets of indisulam. Overall, these results provide insights into the contribution of metabolism to the mechanism of action of indisulam and suggest that metabolic subtypes of tumours may respond differently to therapy. Such knowledge will improve our understanding of which patients would benefit from indisulam treatment.

BIOMEDICAL

SESSION 1: CANCER	Tuesday, June 25	10:15 a.m. – 12:00 p.m.

1D 11:20 a.m. – 11:40 a.m.	Rewiring of energy metabolism drives resistance to the proteasome inhibitor bortezomib

PRESENTING AUTHOR: ***Esther Zaal, Biomolecular Mass Spectrometry and Proteomics, Utrecht University, Netherlands***

CO-AUTHORS: ***Harm-Jan de Grooth, Pieter Langerhorst, Haley Baptist, Wei Wu, Celia R. Berkers***

The proteasome inhibitor bortezomib is successfully applied in the treatment of multiple myeloma, but its efficacy is restricted by the wide-spread occurrence of resistance. Metabolic alterations play an important role in cancer development and aid in the cellular adaptation to pharmacologically changed environments. Metabolic changes may therefore also play an essential role in the development of drug resistance. Interestingly, cells could become reliant on such drug-induced metabolic reprogramming, a vulnerability that could be exploited for therapy. Here, the metabolic pathways involved in resistance to bortezomib were elucidated using a mass spectrometry-based metabolomics approach. To this end, bortezomib-sensitive and -resistant cell lines were profiled using a combination of steady-state metabolomics experiments and stable isotope labelling approaches. These metabolomics studies were complemented with a metabolism-oriented targeted proteomics approach. We demonstrate that BTZ-resistant cells extensively rewire their mitochondrial energy metabolism and identify metabolic drugs that could overcome bortezomib resistance. By investigating gene expression patterns of metabolic genes in genome-wide data of MM patient samples, we show that rewiring of mitochondrial metabolism correlates to drug response and survival. In conclusion, we provide novel mechanistic insights in the role of mitochondrial energy production in mediating bortezomib resistance. Our data indicate that this metabolic rewiring correlates to drug response in MM patients and provide rationale for combining bortezomib with metabolic drugs to increase treatment efficacy.

1E 11:40 a.m. – 11:55 a.m.	Understanding metabolite heterogeneity in pancreatic ductal adenocarcinoma, and the role of adaptive metabolic reprogramming in chemotherapy resistance

PRESENTING AUTHOR: ***Sarah Hancock, University of New South Wales, Australia***

CO-AUTHORS: ***Jesse Estoque, Nigel Turner***

Pancreatic ductal adenocarcinoma (PDAC) has one of the most dismal prognoses in modern medicine with a 5-year survival of just 7.7%. Contributing to the poor survivability of PDAC is its highly aggressive and chemoresistant nature, which is driven by extreme genetic and phenotypic heterogeneity. Mutations in oncogenes result in wide-ranging effects on cell metabolism, allowing the cells to acquire metabolites necessary for rapid growth and proliferation. Cellular heterogeneity can aid the cancer cells in acquiring chemoresistance through adaptive metabolic reprogramming, in which chemotherapy action is circumvented by engaging pro-survival metabolic pathways. In this study, we use an untargeted metabolomics approach to determine the breadth of metabolite heterogeneity across four PDAC cells lines (MiaPaCa2, Panc1, BxPC3 and AsPC1) and one non-cancerous immortalised human pancreatic ductal epithelial (HPDE) cell line. Approximately 800 features (i.e. unique combinations of retention time and mass-to-charge, m/z) were detected across these four pancreatic cell lines and a number of metabolic pathways were found to be altered compared with noncancerous pancreatic cells, including metabolites involved in central carbon metabolism, glycolysis, glutaminolysis, nucleotide synthesis, and lipid synthesis. Metabolite profiling of gemcitabine-treated PDAC cells has uncovered several potential adaptive metabolic pathways, many of which centre around non-essential amino acid metabolism. Work is ongoing to elucidate mechanisms of chemoresistance in PDAC, including the use of stably labelled isotope tracing to further characterise mechanisms of chemoresistance in these cells.

PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

SESSION 2: PLANT APPLICATIONS 1	Tuesday, June 25	10:15 a.m. – 12:00 p.m.

2A Session Keynote
10:15 a.m. – 10:45 a.m.

The volatile flavor composition network in tomato and its modification

PRESENTING AUTHOR: ***Antonio Granell, CSIC, Spain***

Tomato is a model for fruit ripening and more recently also for the identification of the molecular genetic basis of what makes fruit healthy and palatable. Organoleptic and nutritional quality in this climacteric fruit is regulated at different levels and metabolites associated with organoleptic and nutritional composition are part of the ripening process and increasingly the target/focus of geneticists and breeders. The nutritional value of tomato fruit is not based on calories but on the healthy compounds they provide to our diet (main source of antioxidants and vitC in Western diets). Many of the plant/tomato nutritional and healthy compounds are often precursors of compounds that contribute to flavour what supports the contention that good flavour and healthy compounds are part of the mechanism plants use to reward frugivors for dispersing the seed contained in their fruits. Breeding or Biotech approaches aimed to higher nutritional content should go hand with hand with good flavour in order to really have an impact on consumers. In my presentation I will illustrate examples were the use of genetic resources in combination with high throughput genotyping and phenotyping and biotech approaches is contributing to identifying genomic regions, markers and genes associated /underlying the variation in compounds of organoleptic or nutritional value. I will also present how Plant Biotechnology and Synthetic Biology approaches are used in our lab to further expand the natural variation in metabolites either to increase the levels of nutritional or good flavor compounds or to eliminate anti nutritional compounds.

2B 10:45 a.m. – 11:05 a.m.

Developing Advanced and Integrated Metabolomics Technologies to Address the Grand Challenges of Metabolite Identification and Depth of Coverage

PRESENTING AUTHOR: ***Lloyd Sumner, University of Missouri, United States***

CO-AUTHORS: ***Lloyd W. Sumner, Feng Qiu, Dennis Fine, Daniel Wherritt, Zhentian Lei, Anil Bhatia, Mark Schroeder, Aiko Barsch, Sven Meyer***

The vast utility of metabolomics is well documented in the literature; however, its full scientific promise has not yet been realized due to multiple technical challenges. These grand challenges include large-scale confident chemical identification of metabolites and greater depth of coverage. We have developed sophisticated spectral, computational and integrated experimental metabolomics tools for the systematic and biologically directed annotation of plant metabolomes and for greater metabolome depth of coverage. UHPLC-QTOF-MS/MS metabolite profiling was first performed using Medicago truncatula methanol extracts, and the data processed by peak deconvolution and formula prediction. Metabolite identifications were first attempted through spectral matching with custom MS and MS/MS libraries of 222 plant specialized metabolites. Additional identifications were predicted using novel software entitled 'Plant Metabolite Annotation Toolbox' (PlantMAT) that generates in-silico prediction of metabolite structures based upon orthogonal empirical data. PlantMAT includes both GC-MS and LC-MS modules. The data was imported into PlantMAT and structures for approximately 100 saponins and polyphenolic glycosides were predicted. Approximately 80 of these were isolated, purified and concentrated by UHPLC-MS-SPE. The SPE isolated compounds were eluted and 1D and 2D NMR spectra acquired. The results demonstrated that the cumulative platforms allow for higher-throughput and high confidence metabolite identifications. UHPLC-timsTOF-MS/MS analyses were performed to discover potentially coeluting compounds and to increase our metabolomics depth of coverage for isobaric compounds not readily separated by UHPLC. Examples are provided for hydroxylated flavonoids. A library of CCS values are being measured and compiled for added confidence in metabolite identification.

2C 11:05 a.m. – 11:20 a.m.

From MS peak to unambiguous metabolite identification using the WeizMass spectral library and LC-MS-SPE-NMR system

PRESENTING AUTHOR: ***Adam Jozwiak, Weizmann Institute of Science, Israel***

CO-AUTHORS: ***Nir Shahaf, Prashant D. Sonawane, Tali Scherf, Ilana Rogachev, Asaph Aharoni***

Despite rapid advances in MS-based methods, unambiguous and high-confidence metabolite annotation is hitherto a significant concern in metabolomics experiments. One of several complementary approaches that might assist in tackling this intricate issue is the usage of a comprehensive, reference compound-based spectral library. We recently generated the WeizMass LC-MS based library composed of more than 6,000 spectra and representing diverse chemicals structures. We also developed the MatchWeiz software that allows matching of experimental data to the WeizMass library. Using such approach we significantly raised our confidence in metabolite identification, carrying out de-replication of known metabolites in diverse plant species and identifying in high confidence metabolites not reported previously from plants. Direct coupling of LC-MS with Solid Phase Extraction (SPE) and NMR spectroscopy represents a complementary approach. The main advantage of this set-up is its simplicity and low amount of material necessary for metabolite identification. It is especially advantageous in case of amphipathic substances, e.g. saponins, requiring high quantities for NMR analysis. In my presentation, I will demonstrate how hyphenated analytical techniques (i.e. LC-MS-SPE-NMR) facilitate saponin pathway discovery by providing structural data of complex metabolites and generating substrates for enzyme- and bio- activity assays. Combination of high-resolution MS and several 2D-NMR techniques allowed unambiguous identification of novel acetylated medicagenic acid derived saponins from spinach. Finally, we utilized the LC-MS-SPE-NMR system for an unequivocal annotation of biosynthetic intermediates produced by partial reconstruction of the biosynthetic pathway in heterologous system.

SESSION 2: PLANT APPLICATIONS 1	Tuesday, June 25	10:15 a.m. – 12:00 p.m.

2D 11:20 a.m. – 11:40 a.m.	Chocolate metabolomics

PRESENTING AUTHOR: ***Robert Hall, Wageningen UR, Netherlands***

CO-AUTHORS: ***Ric CH de Vos, Isabelle Privat, Maud Lepelley, Roland Mumm, Marco CAM Bink, Jos A Hageman, Jwanro Husson, Veronique Maffone, Philippe Pollien, Jean-Claude Spadone, Susan Strickler, Dominique Crouzillat, Robert D Hall***

Optimising and securing sustainable cacao production is hampered by a lack of knowledge of the chemical and genetic factors controlling the yield and quality of the beans used for preparing chocolate liquor. We have used an integrated, multi-omics approach to genotype and chemically phenotype a dedicated F2 segregating population of a high yielding and a high quality Theobroma cacao parental cross. Fermented beans and cocoa liquors obtained following standard industrial procedures were subjected to comprehensive metabolite profiling (LCMS pos/neg modes, GCTOFMS of (derivatised) polar compounds, and GCMS of volatile components). In parallel, sensory (taste) panel evaluations were performed on the chocolate liquors. Dedicated statistics was applied to model sensory scores based on metabolite profiles and to select a small set of marker compounds predicting sensory quality scores based on either liquor or bean metabolites. Subsequently, genetic mapping of the population was performed using co-dominant molecular markers and this map was used for large-scale Quantitative Trait Loci (QTL) analysis. We identified 1760 traits (metabolites) in beans or liquors and these metabolites concerned many different chemical groups including amino acids, sugars, polyphenols and aromatic components. 484 QTLs were also identified controlling either cocoa quality traits or individual metabolites indicating that 27.5% of the cocoa traits analysed is genetically controlled. Further use of cacao fruit transcriptome data and gene mining of potential candidate genes co-localizing with QTLs controlling key cocoa quality parameters has given us for the first time deep insights into the genetic basis of cocoa (chocolate) quality.

2E 11:40 a.m. – 11:55 a.m.	A tissue specific metabolomic study in hybrid aspen

PRESENTING AUTHOR: ***Ilara Budzinski, Swedish University of Agricultural Sciences (SLU), Sweden***

CO-AUTHORS: ***Ilka N Abreu, Thomas Moritz***

Hybrid aspen is a commercially fast-growing tree, used in short rotation wood production in many countries. As a renewable natural resource, providing timber, fibers and energy, wood is important for the environmental and economic perspective. Wood formation is a complex process, subject to multiple levels of regulation (from cell division through programmed cell death). Despite its importance, the metabolic profile underlying wood formation still poorly understood. To overcome this, we present a tissue specific metabolomics study in hybrid aspen (Populus tremula x P. tremuloides), using cryosectioning. We used four-month-old trees cultivated under greenhouse conditions and supplied with different levels of fertilization solution (low, adequate and high) for controlling growth. The stems were 20 µm thick tangential cryo-sectioned in: bark, inner bark, phloem, expanding phloem, cambium, expanding xylem, xylem and mature xylem. Target and untargeted metabolomics (GC and LC-MS) was performed, including lipidomics analysis. Trees treated with higher fertilization doses showed higher diameter and biomass, compared to the lower treatment. By OPLS-DA we discriminate tissues and treatments. Differentially abundant metabolites (VIP ≥1.0 and $P \leq 0.05$) were identified from bark towards xylem. As an example, proline, rhamnose, trehalose, sucrose exhibited a similar distribution pattern along the tissues, when comparing trees with contrasting stem diameter. However, other metabolites were significant only in trees with smaller (fructose and glucose) or higher (arginine, methionine, salicin, stearyl acid) stem diameter. The results obtained here represent the metabolic overview from bark towards wood-forming tissues, and highlighted the powerful combination of cryosectioning and metabolomics analysis

TECHNOLOGY

SESSION 3: DATA INTEGRATION & DATA BASING 1	Tuesday, June 25	10:15 a.m. – 12:00 p.m.

3A Session Keynote 10:15 a.m. – 10:45 a.m.	The Internet for Social Machines

PRESENTING AUTHOR: ***Barend Mons, GO FAIR, Netherlands***

We are in a transition phase of science, where machines (mainly computers) have become our major research assistants. Humans and computers increasingly work together as 'social machines' to makes sense of complex natural phenomena. However, computers need a very different input as compared to people and the way we adapt the communication and reuse of our research results is adopting to this new situation only at glacial speed. Still, the 15 FAIR Principles, publsihed in 2016, dealing with machine actionable data and services, have found unusually rapid uptake among a broad spectrum of stakeholders, from research scientists who create and reuse data, to publishers who distribute data, to science funders who track impact of data. Barend will describe the FAIR Principles and show examples of how they have been implemented. He will also present a set of core FAIR Metrics that can help gauge the level of FAIRness of any digital resource. Of particular interest is how additional FAIR Metrics can (and should) be defined to address community-specific data structures and analytic requirements. This discussion, and these examples will be presented in the context of the International GO FAIR Initiative. GO FAIR is a voluntary community of stakeholders devoted to implementation solutions of an emerging Internet of FAIR Data and Services.

3B 10:45 a.m. – 11:05 a.m.	TBD

PRESENTING AUTHOR: ***TBD***

CO-AUTHORS: ***TBD***

TBD

3C 11:05 a.m. – 11:20 a.m.	Visualizing metabolomics data in directed biological networks

PRESENTING AUTHOR: ***Denise Slenter, Maastricht University, Netherlands***

CO-AUTHORS: ***Martina Kutmon, Jonathan Mélius, Ryan Miller, Georg Summer, Chris T. Evelo, Egon L. Willighagen***

Metabolomics data describes the state of a biological system at a phenotypic level. Unfortunately, not all measured metabolites can be linked to metabolite identities present in biological pathway models. The resulting sparseness makes it more complicated to use metabolomics data in pathway and network analysis. By creating networks from existing pathways, the metabolic data sparseness can be overcome, by calculating the shortest path between metabolites. To upscale this approach, we need to be able to combine different pathway knowledge bases and introduce detailed directionality information, ensuring the shortest paths follow one-directional biological cause-and-effect paths. The presented work creates subnetworks of shortest, directed pathways between active metabolites. First, with the WikiPathways RDF, we created a directed network of all metabolic reactions from the WikiPathways and Reactome pathway knowledgebase. In the next step, we identified the location(s) of the active metabolites in the network, in which we match data with nodes in the network using knowledge from Wikidata. Finally, using the cyNeo4j app for Cytoscape we extracted the smallest connected subnetwork between the changed metabolites using the functionality of the graph database Neo4j. We developed a new solution to visualize the biological pathways involved in sparse metabolomics data. Using knowledge from two pathway resources, we can show the directed networks between active metabolites from metabolomics data. By using Neo4j and Cytoscape, we ensure the computational calculation environment for larger networks as well as advanced visualization functionality to investigate the identified subnetworks. This approach can be extended with proteomics and transcriptomics data.

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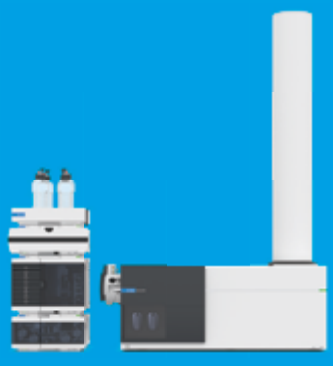

SESSION 3: DATA INTEGRATION & DATA BASING 1	Tuesday, June 25	10:15 a.m. – 12:00 p.m.

3D 11:20 a.m. – 11:40 a.m.	High-throughput metabolomics identifies substrate-enzyme relationships in a metabolism-wide CRISPR interference library

PRESENTING AUTHOR: *Hannes Link, Max Planck Institute for Terrestrial Microbiology, Germany*

CO-AUTHORS: *Stefano Donati, Vanessa Pahl, Timo Glatter*

Construction of large genetic variant libraries has become very fast and versatile. The current challenge is measuring molecular phenotypes of large strain libraries with high-throughput. Here, we show that high-throughput metabolomics identifies metabolic phenotypes in a CRISPR interference library in Escherichia coli. We have recently created a metabolism-wide CRISPR interference (CRISPRi) library that targets all genes in the metabolic network of E. coli and characterized growth of the strains with next generation sequencing (1). Now we measured the metabolome of 118 CRISPRi strains, which have targets in central metabolism, and in biosynthesis pathways of cofactors, nucleotides and amino acids. By using a quantitative and fast (2 min) isotope ratio LC–MS/MS method (2), we quantified about 150 primary metabolites in the CRISPRi strains. In many strains we observed specific increases of substrates of the targeted enzymes. Especially downregulation of enzymes in biosynthetic pathways resulted in an accumulation of intermediates, which are under normal conditions undetectable in E. coli. These substrate-enzyme relationships suggest that biosynthetic enzymes in wild-type cells operate far away from their maximal capacity, and therefore biosynthetic intermediates are often very low abundant. Finally, by integrating the metabolome data with proteome data we show how metabolite concentration changes affect enzyme-level regulation and the global proteome of CRISPRi strains.

3E 11:40 a.m. – 11:55 a.m.	Weighting strategies for the analysis of secondary outcomes in nested case-control metabolomics data

PRESENTING AUTHOR: *Gerard Gonzales, Ghent University, Belgium*

CO-AUTHORS: *Vladimer Kobayashi*

Freely available metabolomics datasets in public repositories allow us to investigate other biological questions without necessarily recruiting new cohorts of human volunteers. However, considering that the data was selected for a different purpose, it may potentially introduce bias when analysing secondary outcomes of interest. We propose to remedy this problem by introducing weights to observations. In this study, we tested various weighting strategies namely, inverse probability weighting and the ones derived from boosting and neural network. We then illustrate their uses to investigate the urinary metabolomic signature specifically associated with smoking by using available data from a case-control urinary metabolomics study where the participants were originally selected for presence or absence of lung cancer (MTBLS28). We show that directly using the dataset without weighting resulted to biomarkers of lung cancer being strongly associated to smoking. This distorts the biological interpretation of metabolites and pathways associated with smoking and does not generalize to new population of non-cancer patients. The combination of weighting strategies with popular analytical models (i.e. PLS-DA, generalized linear models with regularization, and random forest) has the effect of not only leading to statistically valid models with good predictive ability, but also, the extracted biomarkers are independent of the primary outcome. In short, the extracted urinary biomarkers were more specifically associated with smoking than with lung cancer status. Consequently, we developed a framework for introducing weights to guide researchers in choosing an appropriate weighting method. Finally, we discuss the application of the framework to metabolomics studies using secondary data.

NEW FRONTIERS

SESSION 4: NOVEL TECHNOLOGIES	Tuesday, June 25	10:15 a.m. – 12:00 p.m.

4A Session Keynote 10:15 a.m. – 10:45 a.m.	Improving Technologies for High Throughput and Miniaturized Metabolomics for Precision Medicine

PRESENTING AUTHOR: ***Thomas Hankemeier, Leiden University, Netherlands***

Where genomics has proven to be successful to predict disease risk, metabolomics can assess the actual health state and monitor disease development and treatment response. Large-scale metabolomics studies are necessary for better and more specific prognosis of chronic diseases. For this efficient and quantitative metabolomics methods are necessary. Novel technologies to enable this will be discussed in this lecture. We have developed a hanging droplet evaporator that allows innovative workflows for high throughput (HT) and miniaturized sample preparation. We have developed electrodriven sample preparation technologies for metabolomics. Next, the use of microfluidic-based advanced in-vitro models with organotypic characteristics will be introduced, and how this platform can be used to study disease mechanisms and disease pathways identified using metabolomics. This requires miniaturized metabolomics methods and technologies. Actually, similar innovations for HT metabolomics can be used for miniaturized metabolomics. An outlook will be given how metabolomics will impact clinical research and ultimately clinical decision support.

4B 10:45 a.m. – 11:05 a.m.	Investigation of host-microbiota co-metabolism as a new strategy for biomarker discovery – New Chemical Biology tools for Metabolomics analysis

PRESENTING AUTHOR: ***Daniel Globisch, Uppsala University, Sweden***

CO-AUTHORS: ***Mário S.P. Correia, Louis P. Conway, Weifeng Lin, Abhishek Jain, Caroline Ballet, Neeraj Garg***

The detailed investigation of metabolites in human samples has been termed metabolomics and carries a great potential for the discovery of unknown biomarkers. Metabolomics still requires advanced chemical tools compared to other 'omics areas. One of the most exciting scientific developments in the past decade has been the understanding that gut microbiota profoundly impact human physiology. The complex consortium of trillions of microbes possesses a wide range of metabolic activity. Only limited information on this interspecies co-metabolism has been elucidated on a molecular level. We have developed new state-of-the-art Chemical Biology techniques for an enhanced metabolomics analysis using liquid chromatography-coupled with tandem mass spectrometry (UPLC-MS/MS). A unique chemoselective probe immobilized to magnetic beads was prepared for analysis of human fecal samples. This complex probe allows for facile extraction of metabolites and led to increased mass spectrometric sensitivity by a factor of 2000. In another new methods, we utilized selective enzymatic treatment of metabolites in human samples to easily identify converted metabolites and elucidate their chemical formula using mass spectrometry. We chemically synthesized each identified molecule to unequivocally validate the molecular structure. Using this specific workflow, we have successfully identified three times as many sulfated metabolites than reported in the Human Metabolome Database. Our unique metabolite-analyzing methodologies at the interface of Chemistry and Biology are aimed at overcoming limitations in mass spectrometry-based metabolomics research. We are applying these methods for the discovery of unknown metabolites in medical relevant samples to evaluate their potential as biomarkers for pancreatic cancer.

4C 11:05 a.m. – 11:20 a.m.	GC×GC-TOFMS and SIFT-MS approaches for clinical breath-based asthma phenotyping

PRESENTING AUTHOR: ***Pierre-Hugues Stefanuto, Liège University, Belgium***

CO-AUTHORS: ***Delphine Zanella, Joeri Vercammen, Florence Schleich, Renaud Louis, Jean-François Focant***

The ballistic rise of analytical technologies has opened a large playground for all type of "omics" screening. On one side, separation science based on multidimensional methods such as comprehensive two-dimensional gas chromatography (GC×GC) appeared as one of the methods of choice for the characterization complex mixtures. On the other side, direct introduction instruments such as single ion flow tube mass spectrometry (SIFT-MS) offered the capacity to perform both targeted and non-targeted analyses within a few minutes. At the price of high cost equipment and limited adaptability to routine medical usage, GC×GC-HRTOFMS offers the possibility to almost completely characterize a sample composition. This is of prime importance when systems biology are considered. For large scale screening, SIFT-MS can generate compositional patterns from direct sample introduction at the same time than other routine medical actions. These two orthogonal approaches for pathology screening should ideally conduct to identical sample classifications but have never been directly compared over an identical set of patients. In this study, breath from 50 asthmatic patients were analyzed by both techniques. As a reference, asthma phenotypes were established using sputum analysis. Breath samples were collected using TedlarÒ bags. For GC×GC-HRTOFMS analyses, the bags were transferred onto thermal desorption tubes prior to injection. For SIFT-MS, the bags were directly emptied into the instrument. Next, data were analyzed using identical processing workflow. We observed that both approaches offered similar classification capacities. GC×GC-HRTOFMS allowed identifying the putative markers for comparison with previous studies and metabolic interpretation while SIFT-MS offered a faster screening-capacity.

SESSION 4: NOVEL TECHNOLOGIES	Tuesday, June 25	10:15 a.m. – 12:00 p.m.

4D 11:20 a.m. – 11:40 a.m.	Mathematical modelling of metabolism: a driver for developing personalized and precision medicine

PRESENTING AUTHOR: ***Natal van Riel, Eindhoven University of Technology, Netherlands***

Metabolic derailments associated with metabolic syndrome and type 2 diabetes can be studied with mixed meal tests (MTT's). The plasma metabolome enriched with measurements of hormones and cytokines, provide valuable information about the physiological state of an individual. An increasingly important, but complex task is to extract biomedical parameters with diagnostic value from large and multivariate datasets. We applied model-based data processing and analysis, combining computer simulation models, stochastic models of uncertainties and machine learning of time-series data obtained from repeated blood sampling during MTT's. In a mouse model of metabolic syndrome we identified differences in lipid metabolism to be associated with variation in weight gain and development of NAFLD (fatty liver disease). The computational model predicted the progression of dyslipidemia to be linked to bile acids, which was confirmed in a validation study. To investigate the role of bile acids in humans with metabolic syndrome a detailed simulation model of bile acid metabolism and physiology was developed. Model-based analysis of plasma bile acids provides a metabolic 'window' on the gut microbiome and other digestive processes in the gastrointestinal tract. The model was applied to simulate bariatric surgery in patients with metabolic syndrome. The model predicts changes in dynamics in the small intestine to result in a stronger and faster GLP-1 response, hence insulin secretion, explaining observations of rapid glycemic improvement after surgery. The simulation model turned out to be sufficiently robust that personalized variants for individual patients could be made, using MTT plasma bile acid metabolomics as input.

4E 11:40 a.m. – 11:55 a.m.	TIMS and PASEF multiply speed and sensitivity in lipidomics

PRESENTING AUTHOR: ***Catherine G. Vasilopoulou, Max Planck Institute of Biochemistry, Germany***

CO-AUTHORS: ***Karolina Sulek, Andreas-David Brunner, Dmitry Voytik, Aiko Barsch, Sven Meyer, Ulrike Schweiger-Hufnagel, Ningombam Sanjib Meitei, Matthias Mann, Florian Meier***

We have recently introduced a novel scan mode termed 'parallel accumulation – serial fragmentation' (PASEF), which promises to overcome longstanding limitations in throughput and sensitivity of mass spectrometry-based proteomics (Meier et al., PMID:26538118; Meier et al., PMID:6283298). PASEF synchronizes precursor selection with trapped ion mobility spectrometry (TIMS) and achieves over ten-fold increased sequencing rates without loss in sensitivity. Here we explore the benefits of PASEF for lipidomics. We analyzed lipid extracts from human plasma (SRM 1950), mouse liver and HeLa cells via nanoflow LC coupled to a high resolution TIMS-QTOF mass spectrometer (Bruker timsTOF Pro). Raw data were processed with MetaboScape (Bruker) and MS/MS spectra were annotated with SimLipid (PREMIER Biosoft). As compared with conventional TIMS-MS/MS, we acquired six times more MS/MS scans with PASEF, which translated into about twice as many lipid identifications. The PASEF speed allowed us to reduce the LC analysis time from 90 to 30 min, while still covering over 90% of the lipids identified with the longer gradient. Comparison to community efforts reveals an excellent coverage of all major lipid classes from just 1 µL plasma. The ion mobility dimension further allows separation of isomeric species and provides a high-precision measure of lipid collisional cross sections (CCS) with CVs <1%. In a single experiment, we compiled a CCS library of over 1000 lipids and show high correlation with literature as well as predictions based on machine learning (R2>0.98). We anticipate that accurate CCS measurements in conjunction with PASEF acquisition will greatly benefit metabolomics and lipidomics.

SESSION 5: METABOLIC DISEASE	Tuesday, June 25	1:30 p.m. – 3:15 p.m.

5A Session Keynote 1:30 p.m. – 2:00 p.m.	Integrating epidemiologic, pharmacologic, genetic and gut microbiome data in the BBMRI-NL drug-metabolome atlas

PRESENTING AUTHOR: ***Cornelia van Duijn, University of Oxford, United Kingdom***

Progress in high-throughput metabolic profiling provides unprecedented opportunities to obtain insights into the effects of drugs on human metabolism. The Biobanking BioMolecular Research Infrastructure of the Netherlands (BBMRI-NL) has constructed an atlas of drug-metabolome associations for 87 commonly prescribed drugs and 150 clinically relevant plasma-based metabolites assessed by proton nuclear magnetic resonance (1H-NMR). The atlas involves a meta-analysis of ten cohorts (18,873 persons) and uncovers 1,071 drug-metabolome associations after evaluating confounding including age, sex, weight, smoking and co-treatment. Data integration with genetic and pharmaceutical intervention studies on statins shows that cross-sectional data of the BBMRI-NL atlas can yield information of future drug effects. Further data integration involving epidemiological data links proton pump inhibitors to circulating metabolites, biochemical liver-parameters, hepatic steatosis and the gut microbiome. Our atlas provides a tool and starting point for targeted experimental pharmaceutical research to improve drug efficacy, safety and repurposing. We provide a web-based resource for visualisation of the atlas (http://bbmri.researchlumc.nl/atlas/).

5B 2:00 p.m. – 2:20 p.m.	Metabolic profiling of tissue-specific insulin resistance in human obesity: Results from the Diogenes Study and The Maastricht Study

PRESENTING AUTHOR: ***Ilja Arts, Epidemiology & MaCSBio, Maastricht University, Netherlands***

CO-AUTHORS: ***Nicole Vogelzangs, Carla J van der Kallen, Marleen M van Greevenbroek, Birgitta W van der Kolk, Johan WE Jocken, Gijs H Goossens, Nicolaas Schaper, Ronald Henry, Simone J Eussen, Armand Valsesia, Thomas Hankemeier, Arne Astrup, Wim HM Saris, Coen DA Stehouwer, Ellen E Blaak***

Recent evidence indicates that insulin resistance (IR) in obesity may develop independently in different organs, representing different etiologies towards type-2 diabetes and other cardiometabolic diseases. The aim of this study was to investigate whether non-diabetic IR in the liver and in the skeletal muscle are associated with distinct metabolic profiles. Our study included 634 overweight/obese (BMI≥27 kg/m2) adults without diabetes (≤65 years; 63% women) of the European multicenter Diogenes Study. Hepatic insulin resistance index and muscle insulin sensitivity index were derived from a 5-point oral glucose tolerance test (OGTT). Seventeen plasma metabolites were quantified by nuclear-magnetic-resonance spectroscopy. In an independent sample of 540 overweight/obese participants without diabetes (BMI≥27 kg/m2; 40-65 years; 46% women) of The Maastricht Study, 11 metabolites and a 7-point OGTT were available for validation. Replicated results indicate that both liver and muscle IR are associated with elevated levels of (branched-chain) amino acids (isoleucine, alanine), lactate and triglycerides and lower glycine levels, but only liver IR associates with lower ketone body levels (acetoacetate, 3-OH-butyrate) and elevated levels of ketogenic amino acids (leucine, tyrosine), suggestive of decreased ketogenesis. These findings suggest that in early stages of cardiometabolic disease development, distinct metabolic profiles of liver IR and skeletal muscle IR can be observed. This knowledge might enhance developments of more targeted tissue-specific interventions to prevent progression to more severe disease stages.

5C 2:20 p.m. – 2:35 p.m.	Identification of novel metabolites in alkaptonuria by LC-QTOF-MS profiling and flux analysis of a targeted HGD-/- mouse model

PRESENTING AUTHOR: ***Brendan Norman, Institute of Ageing & Chronic Disease, University of Liverpool, United Kingdom***

CO-AUTHORS: ***Brendan P Norman, Juliette H Hughes, Andrew S Davison, Hazel Sutherland, Peter J Wilson, Norman B Roberts, Lakshminarayan R Ranganath, George Bou-Gharios, James A Gallagher***

Alkaptonuria (AKU) is a rare disorder of tyrosine metabolism caused by congenital lack of homogentisate 1,2-dioxygenase (HGD). The primary biochemical consequence of HGD-deficiency is elevated circulating homogentisic acid (HGA), which accumulates in cartilage as 'ochronotic' pigment causing severe early-onset osteoarthropathy. Metabolic profiling was performed to investigate for the first time the wider metabolic consequences of HGD-deficiency in a new targeted HGD-/- mouse model which recapitulates human AKU. Urine from 15 HGD-/- and 14 HGD+/- male age-matched mice was analysed by LC-QTOF-MS. Data were mined using a published accurate-mass/retention-time database developed in-house from metabolite standards with additional compounds of potential interest from wider tyrosine metabolism. Comparing profiles of HGD-/- and HGD+/- urine in negative and positive polarity revealed that the clearest differences (FDR-adjusted $p<0.05$, log2 FC>1.5) were increases in HGA and 7 previously unreported HGA-derived biotransformation products. Other metabolites showing clear alteration in HGD-/- included thymidine-5'-diphospho-alpha-D-glucose, DL-3,4-dihydroxymandelic acid, 2-aminophenol, p-hydroxyphenylacetic acid (increased) malic acid, isocitrate and inosine 5'-monophosphate (decreased). Dehydroxymethylene-HGA, HGA-decarboxylate, HGA-glucuronide and HGA-sulfate were subsequently confirmed as derived from HGA in a flux experiment in which HGD-/- mice were injected with 13C6-labelled HGA; analysis of plasma taken 2-60 min post-injection showed clear evidence of M+6 isotopologues attributable to the 13C6 label. The data indicate previously uncharacterised clearance mechanisms for elevated HGA; the central biochemical cause of morbidity in AKU. Further alteration to urinary metabolites from other pathways including catecholamine, citrate and tryptophan indicate that targeted HGD enzyme disruption has wide-ranging and previously unreported metabolic consequences beyond the tyrosine pathway.

SESSION 5: METABOLIC DISEASE	Tuesday, June 25	1:30 p.m. – 3:15 p.m.

5D 2:35 p.m. – 2:55 p.m.	Combining untargeted metabolomics, human genetics, causal inference, and pathway enrichment to define the obesity metabolome

PRESENTING AUTHOR: *Yu-Han Hsu, Harvard Medical School, United States*

CO-AUTHORS: *Christina M. Astley, Joanne B. Cole, Sailaja Vedantam, Josep M. Mercader, Andres Metspalu, Krista Fischer, Kristen Fortney, Eric K. Morgen, Clicerio Gonzalez, Maria E. Gonzalez, Tonu Esko, Joel N. Hirschhorn*

Obesity is a major health problem associated with extensive metabolic disturbances. Identifying the causal connections between obesity and its associated metabolites can uncover relevant biology and inform intervention strategies. Recent studies have combined metabolite profiling with genetic instrumental variable analyses to infer causality between metabolites, obesity, and related diseases. In this study, we expand upon previous research by using genetic instruments to classify body mass index (BMI)-associated metabolites identified in multiple untargeted metabolomics datasets. Meta-analyses and pathway analyses of both known and unknown metabolites were enabled by our recently developed bioinformatics tool, PAIRUP-MS. We identified several known metabolites that are more likely to be the cause (e.g. alpha-hydroxybutyrate) or the effect (e.g. valine) of higher BMI, or may have a more complex bidirectional cause-effect relationship with BMI (e.g. glycine). Importantly, we also identified about 5 times more unknown than known metabolites in each of these three groups. Pathway analysis including both known and unknown metabolites identified different enriched metabolic pathways in the cause and effect groups, providing further evidence that these metabolite groups are associated with obesity via distinct biological mechanisms. These findings demonstrate the potential utility of our approach to uncover causal connections with obesity from untargeted metabolomics datasets. Combining causal inference using genetic data with the ability to match unknown metabolites across datasets provides a path to jointly analyze many untargeted datasets with obesity phenotypes, which will be required to generate sufficient power for the robust discovery and replication of causal biological relationships between obesity and metabolites.

5E 2:55 p.m. – 3:10 p.m.	Metabolomics of Ndufs4-/- skeletal muscle: adaptive mechanisms converge at the ubiquinone-cycle

PRESENTING AUTHOR: *Karin Terburgh, North-West University, South Africa*

CO-AUTHORS: *Jeremie Zander Lindeque, Shayne William Mason, Francois Hendrikus van der Westhuizen, Roan Louw*

Complex I (CI) deficiency impedes the most efficient mechanism providing electrons to the respiratory chain (RC) — a system that, together with ATP-synthase, produces most cellular energy. Although CI deficiency is the most common mitochondrial defect, it is among the most complex, poorly understood disorders currently lacking an effective treatment. Current research on the whole-body Ndufs4 knockout (Ndufs4-/-) mouse model aims to better understand this disease. Although the model's neurological phenotype has been studied, the effect of CI deficiency on skeletal muscle metabolism remains elusive and despite this tissue's high energetic demand, the phenotype lacks strong muscle involvement. We combined hypothesis-generating metabolic profiling and enzyme assays to gain insight into the metabolism of glycolytic and oxidative skeletal muscles from Ndufs4-/- mice. Multi-platform metabolomics was employed, comprising of liquid chromatography-tandem mass spectrometry, gas chromatography time-of-flight mass spectrometry and proton nuclear magnetic resonance spectroscopy. Enzyme assays revealed an 80% reduction in CI activity in both Ndufs4-/- muscle types, which would greatly reduce electron flux to the RC. As an adaptive response, metabolomics identified that several non-classical pathways participate in an attempt to restore electron flux to CIII via the ubiquinone (Q)-cycle. Among the numerous discriminatory metabolites identified between Ndufs4-/- and wild-type muscles, the most prominent alterations indicate the involvement of the glycerol-3-phosphate shuttle, electron transfer flavoprotein system, CII, and the proline cycle in fuelling the Q-cycle. These adaptive mechanisms could maintain adequate ATP production, despite CI deficiency — providing a possible explanation for the lack of muscle involvement in the Ndufs4-/- phenotype.

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SESSION 6: FOOD APPLICATIONS 1	Tuesday, June 25	1:30 p.m. – 3:15 p.m.

6A Session Keynote
1:30 p.m. – 2:00 p.m.

A metabolomic study on coffee – From coffee brews to liver cancer risk

PRESENTING AUTHOR: ***Augustin Scalbert, International Agency for Research on Cancer (IARC), France***

CO-AUTHORS: ***Joseph A Rothwell, Erikka Loftfield, Pekka Keski-Rahkonen, Nivonirina Robinot, Roland Wedekind, Pietro Ferrari, Callie Kambanis, Neal Freedman, Rashmi Sinha, Augustin Scalbert***

Coffee drinking has been associated with lower risk of certain chronic diseases and overall mortality. In this work, we used a mass spectrometry-based untargeted metabolomic approach (i) to characterize variations in the chemical profiles of 76 coffee brew samples, (ii) to identify biomarkers of coffee intake in 451 participants of the European Prospective Investigation on Cancer and Nutrition (EPIC) study, and (iii) to explore association of coffee-associated metabolites in 1:1 matched case-control studies on liver cancer (n=221 cases) and fatal liver disease (n=242 cases) nested in the ATBC cohort. PCA analyses run on 18 identified coffee compounds showed that the first three principal components were driven respectively by roasting intensity, type of coffee beans and caffeination. In the EPIC study, 8 coffee metabolites identified in serum samples were highly correlated with coffee intake, with trigonelline showing the highest correlation. Differences in the magnitude of correlations were observed between countries, possibly explained by preferences in the types of coffee consumed. In the ATBC cohort, 21 metabolites were jointly associated with coffee intake, liver cancer and liver disease mortality. Trigonellline and six other metabolites (serotonin, leucyl-valyl, 3 glycerophospholipids and hypoxanthine) were inversely associated with liver cancer and liver disease mortality; tyrosine and two bile acids were positively associated with both outcomes. This work adds further support to possible hepatoprotective effects of coffee consumption. It also provides new data on variations of coffee composition and optimal biomarkers of coffee intake important to consider in future epidemiological studies.

6B 2:00 p.m. – 2:20 p.m.

Integrated analysis of metabolomics and microbiome data showing additional dietary effects

PRESENTING AUTHOR: ***Jildau Bouwman, TNO, Netherlands***

CO-AUTHORS: ***Femke Hoevenaars, Everton Souto Lima, Lydia Afman, Martin Beaumont, François Blachier, Sandrine Claus, Annick Hartstra, Louise Kjølbæk, Lesli Hingstrup Larsen, Max Nieuwdorp, Yolanda Sanz, Hans Schött, Kevin Portune, Alfonso Benítez, Bart Keijser, Ben van Ommen***

The burden of life-style related diseases has been increasing since the 70's. Disease prevention through improvement of human health is attracting more and more attention. The microbiome is an important new target in these approaches. Our microbiome affects the metabolism of nutrients and thereby influences host physiology. Currently there is a general lack of understanding about the importance of the role of the gut microbiome in health and well-being. Within the MyNewGut project research is performed on the gut microbiome and its relation to dietary interventions. By integration of the data of separate MyNewGut studies we are expanding the understanding for diet-microbiome-host interaction. Clinical, NMR and microbiome data of 5 different dietary intervention studies were integrated. First all measured parameters (clinical, LC-MS, NMR and microbiome) were aligned to discover overlap between studies. Next, overlapping parameters were selected for further integration. A workflow was developed to align and integrate NMR data the data of the different studies. Also for the microbiome data of all studies a workflow was designed. We show that metabolism is improved by butyrate/FT and fibre/PUFA, but becomes more unhealthy after a high protein intervention.Traditionally microbiome, LC-MS and NMR data is evaluated separately. Integration of all these datasets from the different studies into a model which visualizes treatment effects is useful for detection of new diet-microbiome-host interactions.

6C 2:20 p.m. – 2:35 p.m.

Quantitative Dietary Fingerprinting (QDF)—A Novel Tool for Comprehensive Dietary Assessment Based on Urinary Nutrimetabolomics

PRESENTING AUTHOR: ***Raúl González-Domínguez, Biomarkers and Nutrimetabolomics Laboratory, University of Barcelona, Spain***

CO-AUTHORS: ***Raúl González-Domínguez, Mireia Urpi-Sarda, Olga Jáuregui, Paul W. Needs, Paul A. Kroon, Cristina Andrés-Lacueva***

Accurate dietary assessment is a great challenge in nutritional research, needing powerful and robust tools for the reliable measurement of food intake and monitoring dietary habits. To address this issue, we optimized a novel Quantitative Dietary Fingerprinting (QDF) approach, based on the combination of solid phase extraction and subsequent analysis by ultra-high performance liquid chromatography coupled to tandem mass spectrometry (1). This method was validated for the quantitation of about 350 biomarkers, including (poly)phenolic aglycones, phase II metabolites and microbial-transformed compounds, as well as other dietary components. The simultaneous extraction of the complex urinary food metabolome was accomplished by using Oasis HLB solid-phase extraction plates. To improve the recovery of sulfate conjugates, ammonium formate was added to elution solvents as modifier. Then, chromatographic separation was achieved by reversed-phase UHPLC in very short run times, which facilitates the method application to large sample sizes. Tandem mass spectrometry, by using the multiple reaction monitoring acquisition mode, enabled the selective and sensitive detection of these 350 dietary markers at concentrations usually found in real urine samples. The applicability of this QDF method was assessed by analyzing urine samples collected after acute dietary interventions with various foods, including orange, apple, grapefruit, red wine, beer, coffee, green tea, soy, walnuts and wholegrain bread. Therefore, it could be concluded that the optimized methodology presents a huge potential for the assessment of dietary patterns by means of high-throughput analysis of the urinary metabolomic pattern.

SESSION 6: FOOD APPLICATIONS 1	Tuesday, June 25	1:30 p.m. – 3:15 p.m.

6D 2:35 p.m. – 2:55 p.m.	Grass, beef and human. Unlocking the value of the food chain in New Zealand

PRESENTING AUTHOR: ***Arvind Subbaraj, AgResearch Ltd., New Zealand***

We recently published the ryegrass metabolome and a chemotaxonomic classification of 715 ryegrass genotypes, representing 118 populations from 21 different countries. Ryegrass is integral to temperate pastoral agriculture which contributes to most of the meat and milk production worldwide. Pasture-fed beef is claimed to have several health benefits compared to its grain-fed counterparts. Lipids are central to this debate, and phospholipids are predominantly implicated in the health benefits of meat consumption. We therefore conducted a non-targeted lipidomics analysis of 38 different cuts of pasture-fed Wagyu to account for differences in the lipid profile across the beef carcass. Ten cuts with high phospholipid content were identified, and breed (Wagyu vs Angus) and feed (Pasture vs Grain) effects were evaluated in a separate study. Significant differences in phospholipid content was not observed between pasture-fed Wagyu and Angus. However, phospholipids were in higher concentrations in pasture-fed compared to grain-fed Wagyu beef. Finally, we aim to track the effect of consumption of pasture-fed Wagyu beef, grain-fed beef, and a vegetable-based meat alternative, on circulating lipids and biomarkers of cardiovascular disease risk in humans. An eight-week dietary intervention study is currently underway, and a non-targeted lipidomics analysis of blood plasma will be conducted. This talk will summarise recent metabolomics studies conducted in New Zealand to elucidate and decipher the critical components of the food chain from grass and animal, through to human consumption. Missing links in our understanding e.g. the rumen metabolome, a key component of the grass to animal continuum, will be discussed.

6E 2:55 p.m. – 3:10 p.m.	NMR metabolomics revealed different impact of non-acylated and acylated anthocyanins on plasma metabolic profiles in obese diabetic Zucker rats

PRESENTING AUTHOR: ***Kang Chen, University of Turku, Finland***

CO-AUTHORS: ***Kang Chen, Xuetao Wei, Raghunath Paryjani, Maaria Kortesniemi, Jari Heinonen, Yumei Zhang, Jian Zhang, Tuomo Vainio, Kaisa Linderborg, Baoru Yang***

Anthocyanins are present in food as non-acylated (NAAs) and/or acylated (AAs) and known to possess potent anti-diabetic properties. AAs have been reported to have higher stability, antioxidant activity, and bioavailability compared to NAAs. Currently, no research has been reported comparing the effects of these two forms of anthocyanins on metabolic profile of diabetic models. Here, we aimed to compare the modulatory effects of NAAs (from bilberry) and AAs (from purple potato) on metabolic changes in obese Zucker diabetic rat (fa/fa). Experimental rats were fed with NAAs and AAs at two doses for 8 weeks. 1H NMR metabolomics was used to reveal changes in plasma metabolites. NAAs supplementation reduced the plasma glucose level, but similar effect was not seen in the group fed with AAs. All treatment groups decreased levels of branch chain amino acids (BCAAs) and improved lipid profiles, although no significance was found in the group fed with NAAs at the low dose. AAs fed groups showed improved secretion and sensitivity of insulin, as indicated by the positive change in glutamine/glutamate ratio. In AAs fed groups, decreased levels of lactate, serine, threonine, and glycine might be associated with improved oxidative status and shift in energy production from glycolysis and TCA cycle towards lipid catabolism. Our data demonstrate that NAAs improved glucose level through modulating insulin resistance and lipid profiles. AAs reversed most of the changed levels of metabolites in type 2 diabetes to the normal state by modifying insulin resistance and secretion, oxidative stress, energy production, and lipid profiles.

SESSION 7: FLUX STUDIES	Tuesday, June 25	1:30 p.m. – 3:15 p.m.

7A Session Keynote 1:30 p.m. – 2:00 p.m.	A systems medicine approach to identify new drug targets: Model-driven discovery of metabolic reprogramming in metastatic prostate cancer

PRESENTING AUTHOR: ***Marta Cascante, University of Barcelona, Spain***

CO-AUTHORS: ***Cristina Balcells, Igor marin de Mas, Esther Aguilar, Erika Zodda, Pedro de Atauri, Josep J. Centelles, Balázs Papp, Francesc Mas, Timothy Thomson, Silvia Marin, Marta Cascante***

Tumors harbor combinations of heterogeneous neoplastic cells. In this complex ecosystem, all modalities of mutual cell interactions can take place within the context of environmental cues that exert selective pressures. In spite of the underlying heterogeneity, two broad operational categories of neoplastic cells, namely cancer stem cell (CSC) and non-CSC, are most relevant with regards to two key properties of evolving tumor cells: survival to stress and metastatic colonization. Using a dual model of two clonal subpopulations isolated from an established prostate cancer cell line (PC-3), we have applied a systems biology approach including experimental data integration into genome-scale metabolic models to unveil metabolic differences and potential vulnerabilities to be exploited as putative drug targets. The dual model consists of a CSC-subpopulation (PC-3M-high metastatic potential and low invasiveness) and a non-CSC-subpopulation (PC-3S-expressing EMT markers with high invasiveness and low metastatic potential). Results show that EMT and metastasis programes display different metabolic traits. The main differences observed have been at the level of differential use of glucose and glutamine to fuel TCA cycle, mitochondrial respiration, one-carbon metabolism, beta-oxidation and eicosanoids metabolism. We applied the same approach to identify the metabolic reprogramming associated to platinum resistance and we have also identified metabolic alterations emerging from platinum resistance to be used in combined drug therapies. Acknowledgments: MINECO-European Commission FEDER funds– “Una manera de hacer Europa”(SAF2017-89673-R), AGAUR–Generalitat de Catalunya (2017SGR1033) and Icrea Academia award 2015 granted to MC.

7B 2:00 p.m. – 2:20 p.m.	Using stable-isotope labelled metabolomics to explore cell-cell communication between malaria parasites and host cells

PRESENTING AUTHOR: ***Darren Creek, Monash University, Australia***

CO-AUTHORS: ***Anna Sexton, Christian Doerig, Teresa Carvalho***

Malaria is caused by infection of host red blood cells (RBCs) with the Plasmodium parasite and leads to a range of clinical presentations including fever, anaemia and metabolic acidosis. Characterising the interplay between host and parasite factors is critical for understanding the molecular basis of these clinical symptoms. RBCs are essential for the lifecycle of P. falciparum; however, biochemical changes in uninfected, bystander RBCs have not been studied in detail. We performed an untargeted metabolomics study to investigate metabolic changes in uninfected RBCs when exposed to media from P. falciparum culture (“conditioned RBCs”). We observed significant perturbations to central carbon metabolism in conditioned RBCs. Stable isotope labelled U-13C-glucose was used as a carbon source to differentiate active RBC metabolism from parasite-derived metabolites, and this showed that flux through glycolysis and the pentose phosphate pathway was significantly perturbed in conditioned RBCs. Notably, the level of U-13C-lactate secreted by conditioned RBCs was higher than control cells, indicating increased glycolytic flux. To identify factors driving this effect, P. falciparum culture medium was filtered (to remove protein and extracellular vesicles) and incubated with RBCs. Metabolomic analysis showed that the remaining parasite small molecules, alone, were capable of inducing metabolic perturbations in RBCs. Metabolomic profiling of media from P. falciparum cultures has provided a candidate list of small molecules that may be responsible. Altogether these data demonstrate the modulation of RBC metabolism by P. falciparum, which may contribute to the metabolic symptoms of clinical infection.

7C 2:20 p.m. – 2:35 p.m.	Using deuterium labelled glucose to quantify redox metabolism in plants

PRESENTING AUTHOR: ***Edward Smith, University of Oxford, United Kingdom***

CO-AUTHORS: ***James S O McCullagh, R George Ratcliffe, Nicholas J Kruger***

A complete picture of plant metabolism is essential for understanding plant productivity and successful metabolic engineering. However, a lack of quantitative information about sources of metabolic reductant limits the ability to engineer plants to exploit desirable traits. NADPH is the primary source of cellular reductant, required for biosynthesis and protection against oxidative stress. Whilst the oxidative pentose phosphate pathway represents the most direct route for NADPH production from sugars, there is increasing evidence that other pathways make significant contributions to redox balance. Deuterium based isotopic tracers have recently been developed to quantify the production of NADPH from different pathways in mammalian cells, but the application of these tracers to plants has not previously been explored. In this study LC-MS was used to measure deuterium incorporation into metabolites extracted from heterotrophic Arabidopsis cell cultures grown on [1-^{2}H]glucose or $^{2}H_2O$. The results highlight features of plant metabolism that can confound analysis, such as the interconnectivity of the plant metabolic network, the duplication of pathways in subcellular compartments and the high abundance of enzymes that can catalyse the loss of isotopic label to solvent. Understanding these features is a prerequisite for developing more effective strategies for flux determination in plant metabolism and ensuring data are both quantitative and representative of biological processes in vivo.

SESSION 7: FLUX STUDIES	Tuesday, June 25	1:30 p.m. – 3:15 p.m.

7D 2:35 p.m. – 2:55 p.m.	Modelling Cancer Lipogenesis using REIMS Metabolic Flux analysis in breast cancer cell lines

PRESENTING AUTHOR: *Seyma Turkseven, Imperial College London, United Kingdom*

CO-AUTHORS: *Alvaro Perdones-Montero, Simon Cameron, Nikolaos Koundouros, George Poulogiannis, Zoltan Takats*

Rapid evaporative ionisation mass spectrometry (REIMS) offers a novel opportunity for understanding the alterations in lipid metabolism that plays an important role during cancer progression. REIMS is an ambient technique which allows sample analysis without preparative steps which hold the potential to alter the chemical composition of a sample. Here, REIMS was utilised to monitor metabolic flux during cell proliferation using U-13C-labelled palmitic acid to provide a tracer for lipogenesis in in vitro studies. For labelling experiments, SKBR3, MCF7 and MCF10A cells were seeded into culture dishes using DMEM and DMEM/F12 and were then changed to a fatty acid free-BSA media overnight. Following this, cells were treated with [U-13C]-palmitic acid under normoxic conditions for 6h, detached using a cell scraper, and then analysed using REIMS utilising a CO2 laser for sample heating. The ratio of the labelled palmitate taken up by cells to the production of unlabelled palmitate through de novo lipogenesis showed a similarity in cancer cells and nontumorigenic cells. However, palmitic acid incorporation into complex lipids resulted in specific lipidomic profiles for the different breast cancer cell lines. The detected labelled phospholipids after the supplementation cells with the nutrient might help to further interpretation of the lipid signalling process as the cancer cell lines were related to PI3K/Akt/mTOR mediated regulation of the cell functions. This study demonstrates the applicability of LA-REIMS for the characterization of metabolic flux experiments using labelled metabolites in human cell lines – with wider applications to metabolic flux analysis.

7E 2:55 p.m. – 3:10 p.m.	Exploring the use of GC-CI-MS for stable isotope labeling in metabolomics

PRESENTING AUTHOR: *Jordi Capellades Tomàs, IISPV, Spain*

CO-AUTHORS: *Alexandra Junza, Oscar Yanes*

Isotopic labeling experiments have been incredibly valuable to monitor metabolic reactions in biological systems, which is crucial to understand homeostasis alterations that occur in disease. Experimental determination of metabolic fluxes can be inferred from a characteristic rearrangement of stable isotope tracers (e.g., 13C or 15N) that can be detected by mass spectrometry. Metabolites measured are generally members of well-known metabolic pathways, and most of them can be detected by both gas chromatography (GC) coupled to electron ionization (EI) MS and liquid chromatography (LC) coupled to electrospray (ESI) MS approaches. In here, we show that GC methods coupled to chemical ionization (CI) MS have a clear advantage over alternative methodologies due to GC's superior chromatography separation efficiency and the fact that CI is a soft ionization technique that yields identifiable protonated molecular ion peaks. We tested diverse GC-CI-MS setups, including methane and isobutane reagent gases, triple quadrupole (QqQ) MS in SIM mode or monitoring selected ion clusters using optimized narrow-windows (~10 Da) in scan mode, and standard full scan methods using high resolution GC-TOF and GC-Orbitrap systems. The GC-Orbitrap MS in full scan showed the best performance, enabling precise detection of isotopologues in most metabolic intermediates of central carbon metabolism. Finally, with the aim of overcoming manual operations, we developed an R-based tool called IsoScan that automatically quantifies all isotopologues of intermediate metabolites of glycolysis, TCA cycle, amino acids, pentose phosphate pathway and urea cycle.

SESSION 8: NOVEL INSTRUMENTATIONS, TOOLS AND SERVICES

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SESSION 9: EPIDEMIOLOGY	Tuesday, June 25	3:45 p.m. – 5:30 p.m.

9A Session Keynote
3:45 p.m. – 4:15 p.m.

Integrating metabolomics with genomics, proteomics, and other omics for health and drug research

PRESENTING AUTHOR: ***Karsten Suhre, Weill Cornell Medicine - Qatar, Qatar***

Deep molecular phenotyping of population-based studies allows to investigate disease relevant biological pathway by studying their natural variation in large numbers of individuals. Genome-wide association studies with metabolic endpoints already revealed hundreds of genetically influenced metabotypes that define human metabolic individuality. Recently, these large scale omics studies have been extended by including proteomics and other omics phenotypes, and also by linking these multiomics readouts to epigenetic regulation. In this keynote I will review recent developments in the field and discuss potential applications for health and drug research from a precision medicine's perspective.

9B 4:15 p.m. – 4:35 p.m.

Application of 1H NMR metabolomics in ~7,000 people to investigate potential molecular mechanisms of genetic risk variants for coronary artery diseases

PRESENTING AUTHOR: ***Ibrahim Karaman, Imperial College London, United Kingdom***

CO-AUTHORS: ***Ibrahim Karaman, Gonçalo Graça, Claire Boulangé, Rui Climaco Pinto, Georgia Saylor, Eliana Portilla, He Gao, Nicholette Palmer, Timothy Howard, Chunhong Mao, Donald Bowden, Arfan Ikram, Abbas Dehghan, Ioanna Tzoulaki, Timothy Ebbels, Paul Elliott, David Herrington***

Numerous common genetic variants have been identified for association with the risk of coronary artery disease (CAD) using genome-wide association studies (GWAS). For the vast majority, the molecular mechanisms leading to the disease phenotype are not well understood or are completely unknown. We conducted untargeted and targeted 1H NMR metabolomics to characterize the metabolomic signatures associated with the risk variants to generate new knowledge about possible mechanisms involved in the pathogenesis of CAD. Both CPMG and conventional 1D 1H NMR spectra acquired from serum of participants in the Multi-Ethnic Study of Atherosclerosis (MESA, N=~4,000), the Rotterdam Study (RS, N=~2,000), and the Airwave Health Monitoring Study (Airwave, N=3,000) were used in the untargeted metabolomics analysis. For targeted analysis, we used Bruker Lipoprotein Subclass Analysis (B.I.-LISA) in MESA and Airwave cohorts. The current analyses included 7,141 individuals for CPMG, 7,149 for 1D NMR and 5,794 for B.I.-LISA data. We found that variants in 37 loci out of 365 reported loci for CAD were associated with metabolites at the 1% metabolome-wide significance level. These metabolites mainly originated from lipoprotein subclasses, lipids from several lipoprotein particles, and several amino acids potentially involved in protein synthesis. This information could produce new knowledge on the causal pathways linking genes to cardiovascular diseases and identify novel preventive targets.

9C 4:35 p.m. – 4:50 p.m.

Untargeted metabolomics in a prospective cohort to identify diet-related metabolites associated with age-related cognitive decline

PRESENTING AUTHOR: ***Dorrain Low, Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore***

CO-AUTHORS: ***Sophie Lefèvre-Arbogast, Raúl González-Domínguez, Mireia Urpi-Sarda, Pierre Micheau, Melanie Petera, Delphine Centeno, Stephanie Durand, Estelle Pujos-Guillot, Aniko Korosi, Paul Lucassen, Ludwig Aigner, Cécile Proust-Lima, Boris Hejblum, Catherine Helmer, Cristina Andres-Lacueva, Sandrine Thuret, Cécilia Samieri, Claudine Manach***

With a global rise in ageing population and age-associated diseases, understanding how diet modifies cognitive ageing represents key revenues for prevention. In this discovery (D-CogPlast#) study, we aimed to identify a combination of diet-derived metabolites associated with accelerated cognitive decline using untargeted metabolomics. We leveraged the French Three-City cohort of elderly people and using an exploratory approach, designed a nested case-control study contrasting the metabolic profiles of 209 cases of cognitive decline over 13 years against 209 controls (matched for age, gender and educational level) over 12 years following baseline blood draw. Serum samples were profiled using high-resolution UHPLC-QToF. Validated PLS-DA of the baseline serum profiles clearly distinguished between case and control populations. A signature of 22 serum metabolites were associated with cognitive decline, using bootstrap-enhanced least absolute shrinkage and selection operator (LASSO) regression. Seven metabolites derived from the food metabolome included coffee-derived metabolites (atractyligenin glucuronide, cyclo(leucyl-prolyl) and caffeine), a biomarker of citrus intake (proline betaine), 3-carboxy-4-methyl-5-pentyl-2-furanpropionic acid (CMPFP), a cocoa-derived metabolite (cyclo(prolyl-valyl)) and an unknown compound putatively linked to wine intake. The other endogenous metabolites included three acylcarnitines, glycodeoxycholic acid, a glycerophospholipid, trimethyllysine, glucose, cortisol, creatinine and arginine. The 22 metabolite-signature increased the predictive performance of cognitive decline from a cross-validated Area Under the Receiver Operating Curve of 62% (95% CI 56-67%) to 75% (95% CI 70-80%). Our untargeted metabolomics study supports a role for the food metabolome (e.g., metabolites from coffee, citrus fruits, cocoa and wine) and various alterations in endogenous metabolism in cognitive aging.

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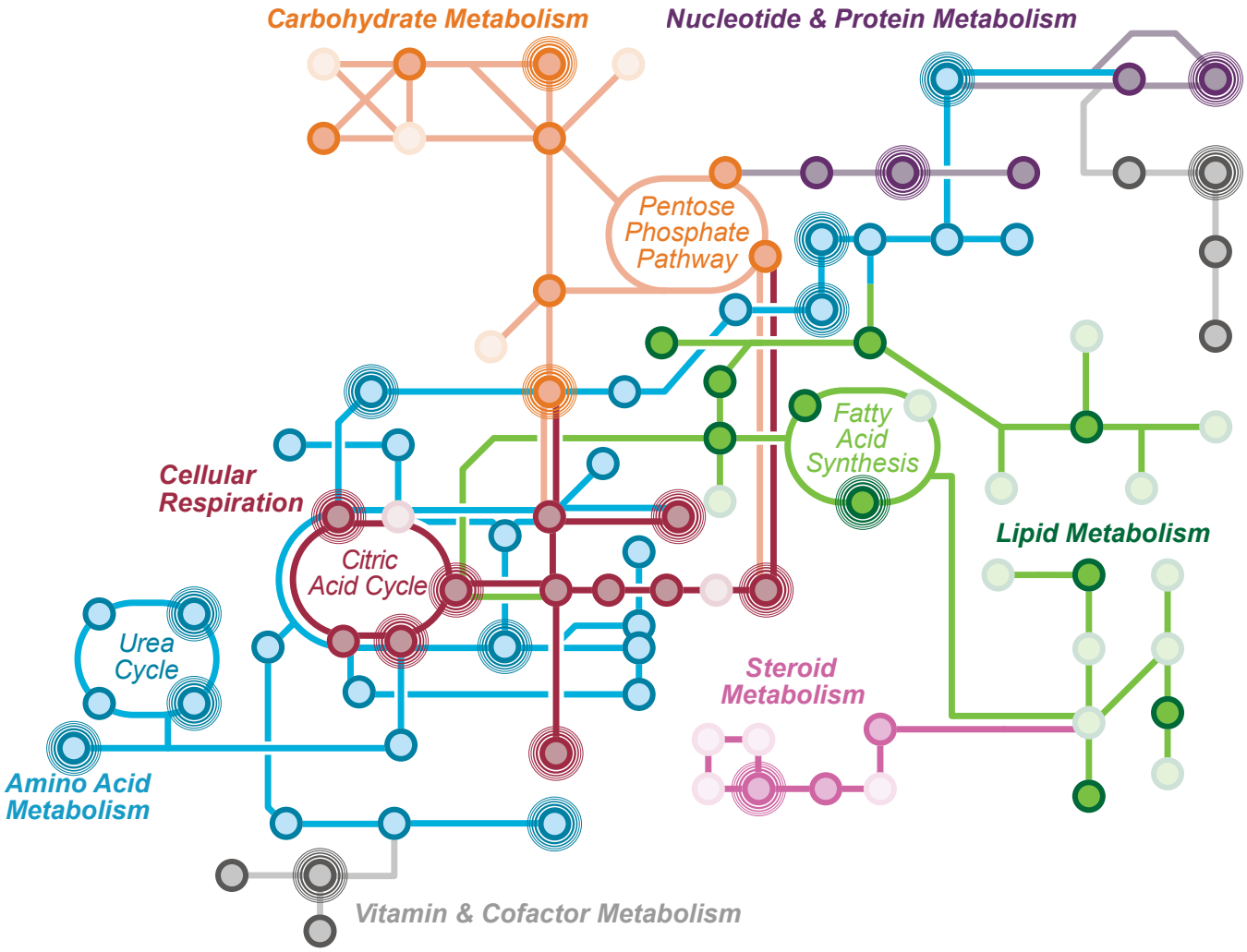

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SESSION 9: EPIDEMIOLOGY	Tuesday, June 25	3:45 p.m. – 5:30 p.m.

9D 4:50 p.m. – 5:10 p.m.	Metabolic profile and the risk of developing prostate cancer - A nested-case control study

PRESENTING AUTHOR: *Ali Moazzami, Department of Molecular Sciences, SLU, Sweden*

CO-AUTHORS: *Hanna E Röhnisch, Cecilie Kyrø, Anja Olsen, Elin Thysell, Göran Hallmans, Ali A. Moazzami*

Prostate cancer is the most frequently diagnosed cancer and the second cause of cancer-related death in men. Identifying modifiable risk factors and markers for disease risk are therefore important. Our aim was to identify metabolites associated with risk of prostate cancer using a nested case-control study design i.e. 777 pairs of prostate cancer cases and their matched controls (n = 1554) recruited from Northern Sweden Health and Disease Study Cohort (NSHDC). Metabolites were quantified with targeted MS and NMR-based metabolomics in fasting plasma samples. Association to disease risk was examined using conditional logistic regression conditioned on matching factors (BMI, age and sample storage time), followed with correcting for multiple testing. Statistical analyses were also done after restriction to non-aggressive and aggressive cases and stratification by baseline age. After correction for multiple testing, we identified a positive association between overall disease risk and plasma levels of two lysophosphatidylcholines (i.e., LPC C17:0 and LPC C18:0). The associations were more pronounced in older subjects (60 years), where the association for LPC C17:0 was significant even after Bonferroni correction. For younger subjects (40 and 50 years), glycine levels positively, and pyruvate levels negatively, associated with risk of overall disease. We also identified positive association between aggressive disease risk and plasma levels of six glycerophospholipids, whereas levels of acylcarnitine C18:2 displayed a negative association. A strong association was found for LPC 17:0 and aggressive disease in older individuals, where individuals in the top quartile had a 3.9 fold higher odds of developing aggressive disease.

9E 5:10 p.m. – 5:25 p.m.	The Metabolome of BMI: A COnsortium of METabolomics Studies (COMETS) Meta-analysis of 85,000 adults

PRESENTING AUTHOR: *Rachel Kelly, HMS and BWH, United States*

CO-AUTHORS: *Steven Moore, Krista Zanetti, Ella Temprosa, Ewy Mathe, Jessica Lasky-Su*

Metabolomics is ideally suited to explore the drivers and consequences of body mass index (BMI) on a mechanistic and metabolic level. Here, we present the largest meta-analysis to date exploring the metabolome of BMI including >85,000 adults from 34 cohorts worldwide; within the context of the COnsortium of METabolomics Studies (COMETS). Of 436 plasma metabolites which could be harmonized across at least ten cohorts; a random-effects meta-analysis identified 186 (47.2%) metabolites as significantly associated with BMI after adjustment for gender, age, race and Bonferroni correction. Of these, 123 increased with increasing BMI, including valine (p=2.4x10-78); tyrosine (p=6.8x10-74) and lactate (p=8.7x10-55); among the top hits. The remaining 63, including cortisol (p=1.3x10-63); and glutamate (p=3.0x10-47) decreased. These results were largely unchanged with additional adjustment for fasting-status, smoking, alcohol consumption, and diabetes status. However, several other BMI-associated metabolites demonstrated significant heterogeneity across the cohorts that was primarily driven by sex and fasting-status. A sub-study of 2000 children (<10years) from three COMETS cohorts additionally determined that different metabolic changes may accompany BMI prior to puberty. These findings confirm the feasibility of large-scale meta-analyses of metabolomics data utilizing the COMETS Analytics platform. Our analyses suggest an important role for branched chained amino acids, cortisol and glutamate metabolism in BMI, which may act through hypothalamic regulation of appetite, insulin sensitivity or dyslipidemia. The results also suggest that the BMI metabolome may differ by sex and between childhood and adulthood, and that BMI-plasma metabolite relationships are sensitive to fasting status at the time of blood draw.

PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

SESSION 10: MICROBIAL APPLICATIONS	Tuesday, June 25	3:45 p.m. – 5:30 p.m.

10A Session Keynote 3:45 p.m. – 4:15 p.m.	Functional characterization of Escherichia coli lipid genes

PRESENTING AUTHOR: *Jos Brouwers, Utrecht University, Netherlands*

CO-AUTHORS: *Aike Jeucken, Chris van de Lest*

The lipidome of a cell is controlled by the intricate actions of lipid gene encoded enzymes. We aimed to increase tolerance of E. coli membranes for hydrophobic compounds by engineering the lipid composition of its membrane. To characterize the contribution of individual genes, we analyzed over 140 strains that either over-express or are knock-out for each individual lipid gene. To this end, we developed a high-throughput procedure that allows for the analysis of over threehundred cell cultures per day. We demonstrate the plasticity of the phospholipidome and discover novel activities of lipid enzymes thanks to the unbiased high-throughput method. We visualize the existance of interesting relationships between lipid classes, lipid species and cell growth. We show that we can reliably model the lipidomic response to exposure to short chain alcohols. Taken together, we present a complete high-throughput lipidomic platform, including growth data, lipid extraction, LC-MS analysis, data processing and visualization.

10B 4:15 p.m. – 4:35 p.m.	Metabolomics-based engineering biology of microorganisms

PRESENTING AUTHOR: *Tomohisa Hasunuma, Engineering Biology Research Center, Kobe University, Japan*

CO-AUTHORS: *Christopher J. Vavricka*

Recent remarkable progresses in DNA sequencing, bioinformatics and synthetic biology have brought various innovations in biotechnology. In constructing microorganisms that can produce functional molecules with high titer, yield and productivity, suitable metabolic pathways and genes can be designed using data stored in database. Variety of microorganisms can be built and then tested by high throughput technologies to learn the relationship between the design and reality with computational approach, which can lead to better design of recombinant microorganisms. The state-of-art Design-Build-Test-Learn (DBTL) cycle would contribute to develop industrial microorganisms in a short time. In the present study, an apparatus for automating pretreatment process of microbial metabolome analysis is developed to enable high throughput and precise analysis of metabolites in Escherichia coli and Sacchayromyces cerevisiae. Our group has recently implemented computationally-deigned novel benzylisoquinoline pathways in E. coli, and used metabolomics to search for clues to overcome loss of key aldehyde intermediates, and a benzylisoquinoline alkaloids (BIA) methylation bottleneck. LC-MS based metabolomics of 115 central metabolites, in addition to BIA related secondary metabolites, resulted in the identification of intermediates related to the recycling of S-adenosyl methionine cofactor as contributors to the methylation bottleneck. In addition, our group has used a CE-MS to understand tolerance of yeast to organic acids, and this could be successfully applied to the optimization of ethanol production from xylose. A later 13C flux and temperature-dependent analysis enabled the optimization of a glycolysis to TCA bottleneck, leading to the highest reported cyanobacteria succinate titer.

10C 4:35 p.m. – 4:50 p.m.	Expanding the Applications of Rapid Evaporative Ionisation Mass Spectrometry (REIMS) to the Pharmaceutical Product Development Workflow

PRESENTING AUTHOR: *Toma Ramonaite, Imperial College London, United Kingdom*

CO-AUTHORS: *Alvaro Perdones-Montero, Kate Alexander-Hardiman, Adam Burke, Andy Ray, Miriam Guest, Simon Cameron, Zoltan Takats*

Rapid evaporative ionization mass spectrometry (REIMS) yields highly specific metabolic profiles of bacteria and fungi which can be used to provide species level taxonomic identification. REIMS offers a novel opportunity for the development of metabolite-based applications for industrial microbiology screening: providing fast and accurate results without the need for pre-analysis preparative steps. One of the focuses of the REIMS applications for pharmaceutical companies is to develop a technique for identifying isolates from environmental monitoring plates which would be an enabler for future real time release (as part of a wider control strategy). Here, we present work on developing REIMS as an automated and high-throughput tool for industrial microbiology with a focus on measuring the metabolomic effect of various growth conditions. Firstly, the effect on bacteria speciation of using refrigerated microbial plates was tested. Samples used for speciation were a mixture of eight frequently found pharmaceutical environmental microorganisms and seven infrequent but concerning clinical isolates. The effect of refrigeration on speciation was tested for 24h, 48h, and 72h incubation at 4°C. Achieved speciation accuracy for regular incubation samples was 97.6% and a measurable effects was only detected after 72 hours of 4°C storage. Secondly, 1 m resolution REIMS imaging was used to identify fungal metabolite distribution over five days growth on live cultures directly from agar culture. Thus, in contrast with other existing techniques (e.g. MALDI) and methods in pharmaceutical companies, REIMS technology could be a technique with substantial savings for business (e.g. time and money, increasing sustainability, integrity and robustness).

SESSION 10: MICROBIAL APPLICATIONS	Tuesday, June 25	3:45 p.m. – 5:30 p.m.

10D 4:50 p.m. – 5:10 p.m.	Linking metabolic function to member taxa in complex microbial communities using metabolome-guided multi-omics

PRESENTING AUTHOR: *Nay Min Min Thaw Saw, Singapore Center for Environmental Life Science Engineering, Singapore*

CO-AUTHORS: *Rogelio E. Zuniga Montanez, Irina Bessarab, Krithika Arumugam, Pipob Suwanchaikasem, Uma Shankari, Mindia A. S. Haryono, Benjamin Kaehler, Lachlan Speirs, Daniela I Drautz-Moses, Yuguang F. Ipsen, Stefan Wuertz, Rohan B. H. Williams*

Whole community metabolic activity is an important phenotypic measure in complex microbial communities (microbiomes) but resolving the identity of taxa who are contributing remains challenging to the presence of multiple taxa that can contribute a given metabolic function. To investigate the feasibility of identifying such inter-relationships, we conducted replicated longitudinal metabolome studies in continuous-culture bioreactors inoculated with activated sludge from a wastewater treatment plant performing enhanced biological phosphorus removal (EBPR) over 19 time points during the P-release/uptake cycle. Extra- and intra-cellular metabolites were extracted by chloroform/methanol/water (2:2:1 v/v) solvent mixture and untargeted metabolomics data were analyzed by ultraperformance liquid chromatography mass spectrometry (UPLC-MS). The community was characterized to the level of metagenome-assembled genomes (Illumina and Nanopore MinION sequencing) and ribosomal-depleted RNA-Seq from the mid-epoch of each phase of the EPBR cycle. We describe a statistical framework, using a functional data analysis approach, for estimating time-varying metabolic profiles associated with physiochemical conditions during EBPR, and from which we can identify known metabolic functions (KEGG reactions) that are 1) plausibly linked to the physico-chemical phenotypes of community and 2) we can link to expressed genes encoding relevant enzymes. For example, in the case of glutamate fed to the reactor, we can identify specific members of phyla Chloroflexi and Actinobacteria that are expressing glutamate related pathways. Our approach enables the systematic analysis and interpretation of multi-omics studies of complex microbial communities using perturbation studies and permits prioritization of targets for testing using NanoSIMS-FISH or related methods.

10E 5:10 p.m. – 5:25 p.m.	Microbial metabolic networks: the hidden key to resilience of coral algal endosymbionts

PRESENTING AUTHOR: *Jennifer Matthews, University of Technology Sydney, Australia*

CO-AUTHORS: *Jennifer L. Matthews, Jean-Baptiste Raina, Justin R. Seymour, David J. Suggett*

The intimate relationship between reef-building corals and their associated microorganisms is fundamental to healthy coral reef ecosystems. Endosymbiotic microalgae (Family: Symbiodiniaceae) and coral-associated microbes support coral health and resilience through metabolite transfer, interpartner signalling, and genetic exchange. Much of our understanding of the coral holobiont relationship has come from studies that investigated either coral-Symbiodiniaceae or coral-microbial interactions. However, the inherent ecological and metabolic interactions occurring between Symbiodiniaceae and other microbes has been almost entirely overlooked. Recent evidence of intimate ecological coupling between phytoplankton and bacteria has demonstrated that obligate resource exchange with microbes underpins the ecological success of marine microalgae. We hypothesize that similar associations with microbial consortia regulate Symbiodiniaceae fitness and that this in turn will govern the health of corals. Using gas chromatography–mass spectrometry (GCMS), we compared the metabolite profiles and physiology of Symbiodiniaceae cultures at increasing levels of axenisation to investigate how associated microbes regulate Symbiodiniaceae fitness. We then applied 13C stable-isotope labelling coupled to GC-MS to characterise carbon translocation from associated microbes to Symbiodiniaceae. Our data indicate that microbes act as resource surrogates for Symbiodiniaceae when they live transiently as free-living cells outside of their host corals. Mapping carbon fate reveals vital functions that associated microbes contribute to Symbiodiniaceae hosts, including molecular signalling pathways and amino acid metabolism. Unravelling the significance of the microbial consortium on Symbiodiniaceae fitness provides a step-change in thinking for the resilience of coral reef organisms to survive in a changing ocean, with wider applications for understanding algal-bacterial interactions in extreme environments.

SESSION 11: METABOLITE IDENTIFICATION 1	Tuesday, June 25	3:45 p.m. – 5:30 p.m.

11A Session Keynote 3:45 p.m. – 4:15 p.m.	Infrared ion spectroscopy: new opportunities for molecular structure identification in MS-based metabolomics

PRESENTING AUTHOR: ***Jos Oomens, Radboud University, Netherlands***

CO-AUTHORS: ***Jonathan Martens, Giel Berden, Rianne van Outersterp, Kas Houthuijs, Karlien Koene, Leo Kluijtmans, Udo Engelke, Ron Wevers***

Studies in metabolomics often rely strongly on mass spectrometry (MS), enabling the sensitive, mass-resolved detection of metabolites in complex mixtures, such as patient samples. The primary weakness of MS-based methods is the limited molecular structure information that is provided even by accurate mass values. Despite the availability of extensive MS/MS and LCMS databases, it remains challenging to distinguish between closely related structures (e.g. positional or stereo isomers), let alone to identify unknowns not present in databases. We have developed methods that allow one to record an infrared (IR) spectrum for species in situ isolated in an ion trap mass spectrometer. Infrared ion spectroscopy (IRIS) is based on the coupling between a frequency-tunable IR laser source and the mass spectrometer. The IR spectrum provides structural information on the connectivity between the constituent atoms of a molecule that can be derived on the basis of reference IR spectra, either experimental or theoretical. IRIS thus combines the selectivity of MS with the structural diagnostics of IR spectroscopy. We will present proof-of-principle experiments, but also actual identification of previously unknown metabolites that can serve as new biomarkers for an inborn error of metabolism. We will show that IRIS is fully compatible with existing MS workflows in metabolomics and especially with liquid chromatography mass spectrometry (LCMS). Furthermore, the ability to accurately predict IR spectra on the basis of quantum-chemical calculations – in strong contrast to the situation for MS/MS spectra – opens new venues towards efficient reference-free identification.

11B 4:15 p.m. – 4:35 p.m.	mFam – prediction of metabolite families based on spectral data

PRESENTING AUTHOR: ***Hendrik Treutler, Dept. of Biochemistry of Plant Interactions, Leibniz Institute of Plant Biochemistry, Germany***

CO-AUTHORS: ***Asaph Aharoni, Pierre-Marie Allard, Megan Augustin, Ulschan Bathe, David Broadhurst, Corey Broeckling, Joerg Buescher, Stefanie Doell, Oliver Fiehn, Maximilian Frey, Andrej Frolov, Emmanuel Gaquerel, Geert Goeminne, Alain Goossens, Jérémy Grosjean, Maria Halabalaki, Stephanie Herman, Justin van der Hooft, Kim Kultima, Toni Kutschan, Romain Larbat, Tytus Mak, Jacob Pollier, Stacey Reinke, Nir Shahaf, Amy Sheflin, Alena Soboleva, Otmar Spring, Lloyd Sumner, Ric De Vos, Jean-Luc Wolfender, Gerd Balcke, Steffen Neumann***

Using untargeted approaches, modern hyphenated MS instrumentation is capable of generating thousands of mass to retention time features at the MS1 and the MS/MS level. The full structure elucidation of all features from a biological sample set is unfeasible and annotations at the level of metabolite families help to interpret spectral data sets and thus answer biological questions (van der Hooft et al. 2016, Wang et al. 2016). We have developed mFam to identify conserved MS/MS fragmentation patterns of metabolite families. Analogously, pFam automatically classifies protein families based on conserved sequence domains in proteomics. mFam aims at the automatic annotation of MS/MS spectra of unknown compounds at the metabolite family level. We mined >500,000 spectra of >25,000 compounds from different HR MS/MS spectral libraries. We classified these compounds into >1,000 metabolite families based on the ChemOnt chemical taxonomy (Feunang et al. 2016). For hundreds of metabolite families we extracted characteristic MS/MS fragmentation patterns and built a library of predictive classifiers. Using MetFamily we demonstrate the application of mFam to predict metabolite families in untargeted metabolomics experiments with promising results (Treutler et al. 2016). Since important metabolite families such as sesquiterpene lactones are still sparsely populated in available spectral libraries the predictivity of those classifiers can be limited. We launched the mFam initiative to broaden the coverage of those metabolite families. By now we jointly collected >6,000 HR MS/MS spectra including spectra for ~100 sesquiterpene lactones. These spectra will be added to MassBank in due course and we welcome further contributions.

11C 4:35 p.m. – 4:50 p.m.	Supercharging Comparative Metabolomics with METABOseek

PRESENTING AUTHOR: ***Maximilian Helf, Cornell University / Boyce Thompson Institute, United States***

CO-AUTHORS: ***Frank C. Schroeder***

Comparative analysis of high resolution mass spectrometry (HRMS) data plays a central role for our understanding of living systems, including elucidation of metabolic networks and small molecule biosynthetic routes. However, data processing is presenting a productivity bottleneck, preventing widespread adoption of comparative metabolomics as a routine tool to complement transcriptomics. To break this barrier, we developed METABOseek, an R/shiny-based, discovery-oriented platform to extract differential features from HPLC-HRMS data. Integrated analyses include xcms-based feature detection, statistical analysis, prediction of molecular formulas, annotation of MS2 spectra, MS2 molecular networking and chemical compound database searches. By making metabolomics tools more accessible, METABOseek enables high-throughput identification of differential molecular features and facilitates structure elucidation of novel compounds, addressing an urgent need to explore the vast universe of unknown compounds revealed by HPLC-HRMS. METABOseek also serves as a launch pad for innovative data analysis strategies developed by the metabolomics community. Many new software tools do not include a graphical user interface and require users to write scripts for their data analysis. This generates a growing disconnect between the available data analysis tools and their potential user base: getting to know the functions and data standards used in a tool takes time and keeps users from integrating them into their workflows, especially when programming experience is limited. The open-source, modular architecture of METABOseek allows developers to dramatically expand their audience by integrating script-based analysis tools into its graphical user interface with minimal effort. METABOseek is available for download and as an online preview version at https://metaboseek.com.

SESSION 11: METABOLITE IDENTIFICATION 1	Tuesday, June 25	3:45 p.m. – 5:30 p.m.

11D 4:50 p.m. – 5:10 p.m.	Ion mobility – mass spectrometry based multi-dimensional metabolite annotation

PRESENTING AUTHOR: *Zhiwei Zhou, IRCBC, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, China*

CO-AUTHORS: *Zhengjiang Zhu*

Unambiguous identification of metabolites is the long-standing challenge for untargeted metabolomics. Ion mobility – mass spectrometry (IM-MS) shows great potential for metabolomics application due to its excellent capability for both separation and metabolite annotation. Although the collision cross section (CCS) value derived from IM-MS has been widely proved to improve the metabolite annotation, it still lacks effective and universal ways to integrate CCS values with other available descriptors (e.g. MS/MS spectra) to provide confident metabolite annotation. To address this challenge, we present the first strategy to integrate CCS values with in-silico MS/MS spectra to perform accurate metabolite annotation. Here, we first optimized the CCS value prediction algorithm with a large and complex training set (containing ~2000 CCS values). It significantly expanded the applicable coverage for prediction, and enabled to generate large-scale CCS values for metabolites. These predicted CCS values were further integrated with in-silico MS/MS spectra (generated by CFM-ID) to perform confident metabolite annotation. Comparing with using exact mass match, this strategy was validated to effectively reduce ~30-40% false candidates for different database match (e.g. KEGG, MINE) using several benchmark datasets. It also supported to improve the rank of correct metabolite comparing with directly using in-silico MS/MS spectra. In addition, we would demonstrate the discrimination power of integrating CCS and MS/MS spectra in the whole metabolome level. In conclusion, the strategy of integrating multi-dimensional properties (i.e. m/z CCS and MS/MS spectrum) effectively promoted metabolite annotation.

11E 5:10 p.m. – 5:25 p.m.	Recommending substructures for unknown tandem mass spectra

PRESENTING AUTHOR: *Youzhong LIU, University of Antwerp, Belgium*

CO-AUTHORS: *Liu Youzhong, Mrzic Aida, Meysman Pieter, De Vijlder Thomas, Edwin P. Romijn, Valkenborg Dirk, Bittremieux Wout, Laukens Kris*

Structural elucidation of unknown metabolites from LC-MS/MS spectra remains a challenging task. However, these metabolites may share common substructures, which may result in common spectral features (product ions and mass differences). Through molecular networking (GNPS) and mass motif analysis (MS2LDA), an increasing number of spectral patterns have been discovered in high-quality spectral libraries. However, the lack of automated structure annotation system prevents these patterns from being useful for partial identification of unknown metabolites and further biological interpretation. We present a method that mine patterns containing both spectral features and substructures from spectral libraries. These novel patterns are represented as “rules” that associate a substructure with single or several spectral features. Most rules represent high sensitivity for substructure prediction, and they have two important characteristics: i) Spectral features are error-free theoretical masses and mass difference; ii) Predicted substructures are maximum common substructures (MCSs) between biological molecules – non-informative substructures are minimized. From GNPS spectral library, we generated 8378 rules that associate spectral features to 712 substructures, thus recommending substructures in an automated fashion. We validated GNPS rules on both expert-annotated spectral patterns (MS2LDA motifs) and independent test spectra from CASMI challenge, both showing a good agreement with the ground-truth. Using non-targeted MS/MS data of yeast, we implemented a system biology approach that clusters metabolites sharing substructure, revealing substructure enrichment in two yeast phenotypes. Our goal is to achieve system biology interpretation of massive MS/MS data without identifying every metabolite. Our substructure recommendation system was developed into an openly-available web-tool at messar.biodatamining.be.

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NEW FRONTIERS

SESSION 12: STEM CELLS/ORGANOIDS/ORGAN-ON-A-CHIP	Tuesday, June 25	3:45 p.m. – 5:30 p.m.

12A Session Keynote 3:45 p.m. – 4:15 p.m.	No transporters means no transport – and assessment of the 'real' (natural) substrates of xenobiotic transporters.

PRESENTING AUTHOR: ***Douglas Kell, University of Liverpool, United Kingdom***

CO-AUTHORS: ***Steve O'Hagan***

Despite what it says in textbooks, bilayer flux through intact cell membranes is negligible [1] and hydrophobicity is often a poor guide to permeability. Often their 'natural' substrates are unknown. We compare several molecular fingerprint encodings for marketed, small molecule drugs, and assess how their rank order varies with the fingerprint in terms of the Tanimoto similarity to the most similar endogenous human metabolite as taken from Recon2. For the great majority of drugs, the rank order varies very greatly depending on the encoding used, and also somewhat when the Tanimoto similarity (TS) is replaced by the Tversky similarity. However, for a subset of such drugs, amounting to some 10% of the set and a Tanimoto similarity of ~0.8 or greater, the similarity coefficient is relatively robust to the encoding used. This leads to a metric that, while arbitrary, suggests that a Tanimoto similarity of 0.75-0.8 or greater genuinely does imply a considerable structural similarity of two molecules in the drug-endogenite space. However, the majority are in fact similar to natural products [2]

12B 4:15 p.m. – 4:35 p.m.	Spatial Isotope tracer Metabolomics to study 13C labelled Metabolite Distribution in 3D Tumor Spheroid Cell culture

PRESENTING AUTHOR: ***Prasad Phapale, EMBL, Germany***

CO-AUTHORS: ***Mariia Naumenko, Karin Mitosch, Theodore Alexandrov***

Tracer-based studies when performed in 2D cell culture do not provide information about spatial aspects of metabolism neglecting spatio-molecular gradients and 3D microenvironment manifested in tissues and tumors. Multicellular tumor spheroids in 3D cell cultures are established in vitro models to mimic the primary tumors. Study of the distribution of isotope-labeled metabolites in spheroids can give us a valuable spatial understanding of the 3D spatial aspects of tumor metabolism. Here we present a method combining using 13C6-glucose as a tracer in 3D cell culture and untargeted LC-MS/MS analysis to map central carbon metabolism spatially across different layers of spheroids. Tumor spheroids were cultured using HCT116 human colon carcinoma cells for 10 days in Nunclon SpheraTM plates. Metabolites and lipids were extracted from each layer as well as from respective culturing medium to perform untargeted metabolomic profiling using our high-resolution LC-MS/MS-based EMBL-MCF platform (protocols available online https://www.embl.de/mcf/metabolomics-core-facility/protocols). Preliminary results of the metabolite profiling show the highest incorporation of the 13C-label in layer 1 and the lowest in layer 3. The numbers of carbons labeled for pyruvate, succinate, sugar phosphates, other glycolytic metabolites, and amino acids were found to be decreasing from layer 1 to layer 3. The necrotic core with dead cells had the least incorporation of the 13C label which suggests diffusion of labeled metabolites across layers. Our results suggest that the developed method is useful for investigating spatial aspects of metabolic fluxes in a tumor spheroid model.

12C 4:35 p.m. – 4:50 p.m.	Microengineered Human Blood Vessel for Next Generation Drug Discovery

PRESENTING AUTHOR: ***Abidemi Junaid, Systems Biomedicine and Pharmacology, LACDR, Leiden University, Netherlands***

CO-AUTHORS: ***W. Stam, J.M. van Gils, C. van Kooten, S.C. Dólleman, H.C. de Boer, V. van Duinen, A. Mashaghi, A. J. van Zonneveld, T. Hankemeier***

Chronic kidney disease (CKD) is associated with cardiovascular complications including heart failure. To identify early prognostic factors for heart failure in CKD, we aim to explore the association of circulating plasma factors with microvascular integrity. As current human 2D models with cultured endothelial cells lack sufficient complexity to assess the functionality of microvascular endothelial-pericyte interactions, research on microvascular loss largely depends on pre-clinical animal models for ischemia/reperfusion injury. We recently developed a microfluidics-based, 3D 'microvessel-on-a-chip' platform that models patient specific human microvessels and allows quantitative and parallel testing of microvascular leakage. Human umbilical vein endothelial cells (HUVECs) were cultured to generate microvessels-on-a-chip. To assess microvascular destabilization, the layout of the microfluidics platform was modified to measure microvascular leakage of fluorescently labelled albumin by means of high-resolution time-lapse fluorescent microscopy. In this small volume platform, we were able to measure metabolites that are critical in disease progression by using liquid chromatography–tandem mass spectrometry metabolomics. Additionally, we developed an approach to test plasma samples of patients suffering from vascular diseases for the presence of destabilizing factors such as thrombin or TNF-alpha. Our platform may serve as a unique tool for microvascular destabilization studies as well as for the development of novel therapeutic strategies to combat cardiovascular complications. Plasma samples of patients with a destabilizing profile may well predict ongoing microvascular rarefaction and risk of cardiovascular complications.

SESSION 12: STEM CELLS/ORGANOIDS/ORGAN-ON-A-CHIP	Tuesday, June 25	3:45 p.m. – 5:30 p.m.

12D 4:50 p.m. – 5:10 p.m.	Stable Isotope-Resolved Metabolomics (SIRM) Defines DEK Oncogene Driven Metabolic Reprogramming in 3D Epidermal Organoids

PRESENTING AUTHOR: ***Sara Vicente-Muñoz, NMR-Based Metabolomics Core Facility, Division of Pathology and Laboratory Medicine, United States***

CO-AUTHORS: ***Sara Vicente-Muñoz, Marie C Matrka, Miki Watanabe, Marion G Brusadelli, Kaylin Earnest, Andrew N Lane, Lindsey E Romick-Rosendale, Susanne I Wells***

The DEK oncoprotein is amplified and overexpressed in many malignancies, including squamous cell carcinomas (SCCs) originating in keratinocytes of the human epidermis. Our previous work on 2D cell models demonstrated that DEK overexpression reprogrammed metabolism at the level of aerobic glycolysis and oxidative phosphorylation in the absence of proliferative gains, indicating that DEK drives metabolic reprogramming. Engineered 3D epidermal organoids, which mimic stratified human epidermis, show induction of hyperplasia by DEK overexpression and a significant shift in steady state metabolism. To reconstruct global metabolic networks in 3D models of human epidermis, we report the application of stable isotope-resolved metabolomics (SIRM) on epidermal organoids. The goal was to unequivocally define DEK-driven metabolic pathways and specific enzymes, and their respective roles in enabling and sustaining oncogenic phenotypes. Normal immortalized keratinocytes were transduced with either control or DEK overexpression vector, placed into 3D organoid culture conditions for 13 days, and then incubated with 13C6-glucose for an additional 24 hrs prior to collection and metabolite extraction. NMR spectroscopy was used to determine positional isotopomers in downstream metabolites. Media aliquots were collected post isotope addition to quantify 13C6-glucose consumption and de novo production of labelled metabolites. Incorporation of 13C6-glucose validated SIRM feasibility in this system; we observed numerous 13C-labeled metabolites including lactate, alanine, glutamine and glutamate. Comprehensive analyses of DEK-overexpressing versus control organoids defined a number of reprogrammed pathways including glycolysis, the TCA cycle and glutathione metabolism. Current experiments aim to target therapeutically relevant enzymes for the prevention of DEK-dependent SCC development and progression.

12E 5:10 p.m. – 5:25 p.m.	Metabolomics as a quality control for the production of chondrogenic microtissues towards characterized endochondral bone regeneration

PRESENTING AUTHOR: ***Niki Loverdou, Prometheus, Division of Skeletal Tissue Engineering, KULeuven, Belgium***

CO-AUTHORS: ***G. Nilsson Hall, K.Bernaerts, B.Ghesquiere, G.Carmeliet, I.Papantoniou, L.Geris***

The use of 3D microtissues is becoming a standard for bone tissue engineering approaches, as this format allows cell-cell and cell-extracellular matrix interactions. Considering the role of metabolism as a key regulator of stem cell fate and the high sensitivity of metabolomics, this study aims to identify metabolic quality attributes indicative of a functional cartilage intermediate TE construct. LC-MS (liquid chromatography-mass spectrometry) tracer analysis was conducted to investigate metabolic alterations during chondrogenic differentiation of spheroids of hPDCs (human periosteum derived stem cells). 13C labeled glucose, glutamine but also serine and aspartate have been used, as these metabolites showed significant differences between the time points of interest in a prior exometabolomics study. Samples were analyzed at day 0, day 14 and day 21, time points indicative of the proliferating, prehypertrophic and hypertrophic state. Our tracer analysis results showed progressive 13C glucose enrichment in palmitate from 0% at day 0 to 8% at day 14 and 22% at day 21, suggesting activation of fatty acid synthesis. Furthermore, we observed 13C glutamine enrichment in proline from 0 % at day 0 to 20 % at day 14 and 42,5 % at day 21 and a similar trend of 13C glutamine contribution to hydroxyproline (from 0 % at day 0 to 35 % at day 14 and 38 % at day 21). The consecutive stages of chondrogenic differentiation of hPDCs are characterized by specific metabolic adaptations and highlight the importance of unexplored metabolic pathways such as fatty acid and glutamine metabolism for chondrogenic differentiation.

SESSION 13: LIPIDOMICS AND CARDIOVASCULAR DISEASE | Wednesday, June 26 | 10:15 a.m. – 12:00 p.m.

13A Session Keynote
10:15 a.m. – 10:45 a.m.

High-throughput lipidomic quantitation of human blood in cancer screening

PRESENTING AUTHOR: *Michal Holčapek, University of Pardubice, Czech Republic*

CO-AUTHORS: *Michal Holčapek, Denise Wolrab, Robert Jirásko, Michaela Chocholoušková, Ondřej Peterka, David Vrána, Bohuslav Melichar, Roman Hrstka*

A large diversity of lipids is found in eukaryotic cells, where they fulfill important physiological functions. The dysregulation of lipids is often related to serious diseases, e.g., various types of cancer. The robust, high-throughput, and validated analytical methods for the lipidomic quantitation can be applied for biomarker discovery research and also for monitoring the progress of disease therapy. MS and its coupling with the liquid-phase separation techniques together with exogenous internal standards is the most common approach for the lipidomic quantitation. We have developed the following MS based methods for the high-throughput clinical lipidomic quantitation: 1/ shotgun ESI-MS using characteristic NL and PI scans on QqQ, 2/ UHPSFC/MS, 3/ UHPLC/MS, and 3/ MALDI coupled to Orbitrap mass analyzer. Shotgun MS, UHPSFC/MS, and UHPLC/MS techniques are applied mainly for glycerophospholipids, sphingolipids, and glycerolipids using positive-ion ESI, while MALDI is used in the negative-ion mode to obtain complementary information on sulfatides and other anionic lipid subclasses. About 300 – 500 lipid species are typically quantified in studied biological samples from over 1000 human subjects, mainly plasma or serum from healthy volunteers and cancer patients. All mentioned methods follow the basic rule of reliable lipidomic quantitation that IS should be coionized with analytes from the same lipid subclass. Finally, MDA methods are applied for building the statistical models to differentiate cancer patients and healthy controls with over 90% accuracy for samples with known classification and also for blinded samples. This work was supported by project No. 18-12204S sponsored by the Czech Science Foundation.

13B 10:45 a.m. – 11:05 a.m.

Changes in plasma lipids predict pravastatin efficacy in secondary prevention

PRESENTING AUTHOR: *Peter Meikle, Baker Heart and Diabetes Institute, Australia*

CO-AUTHORS: *Kaushala S Jayawardana, Piyushkumar A Mundra, Corey Giles, Christopher K Barlow, Paul J Nestel, Elizabeth H Barnes, Adrienne Kirby, Peter Thompson, David R Sullivan, Zahir H Alshehry, Natalie A Mellett, Kevin Huynh, Malcolm J McConville, Sophia Zoungas, Graham S Hillis, John Chalmers, Mark Woodward, Ian C Marschner, Gerard Wong, Bronwyn A Kingwell, John Simes, Andrew M Tonkin*

Background- Statins have pleotropic effects on lipid metabolism. However, the relationship between these effects and future cardiovascular events is unknown. We sought to characterise the changes in lipid species upon pravastatin treatment and define the relationship with risk reduction for future cardiovascular events. Methods - Plasma lipids (n=342) were measured on baseline and one-year follow-up samples from a LIPID study sub-cohort (n=4991). The associations of changes in lipids with treatment and cardiovascular outcomes were investigated using linear regression and cox regression. The effect of treatment on future cardiovascular outcomes was examined by the relative risk reduction (RRR). Results - Pravastatin treatment was associated with changes in 206 lipids. Species containing arachidonic acid were positively associated while phosphatidylinositol species were negatively associated with pravastatin treatment. Findings were replicated in an ADVANCE case-cohort. A greater decrease and increase in the phosphatidylinositol (PI(36:2)) and phosphatidylcholine (PC(38:4)) respectively, were associated with fewer events. The RRR from pravastatin treatment for cardiovascular events reduced from 23.5% to 16.6% after adjustment for clinical risk factors and change in LDL-C, and to 3.0% after further adjustment for the change in the lipid ratio PI(36:2)/PC(38:4). 58% of the treatment effect was mediated by the change in PI(36:2)/PC(38:4), as indicated by causal mediation analysis. Stratification of patients into quartiles of change in PI(36:2)/PC(38:4) indicated no benefit of pravastatin in Q4. Conclusion-The change in PI(36:2)/PC(38:4) predicts benefit from pravastatin, independent of change in LDL-C, demonstrating its potential as a biomarker for monitoring the clinical benefit of statin treatment in secondary prevention.

13C 11:05 a.m. – 11:20 a.m.

Metabolomics analysis of human atherosclerotic plaques reveals a potential novel pathway of macrophage foam cell apoptosis in advanced atherosclerosis

PRESENTING AUTHOR: *Panagiotis Vorkas, Imperial College London, United Kingdom*

CO-AUTHORS: *Sarah Onida, Kevin Woollard, Alun H Davies, Elaine Holmes*

Atherosclerosis remains a leading worldwide cause of mortality and morbidity. In this study, ultra-high performance liquid chromatography coupled to mass spectrometry (UHPLC-MS)-based metabolomics were utilized for the analysis of human advanced atherosclerotic tissue. From 78 patients, a total of 52 carotid and 26 femoral plaques were compared to 16 adjacent arterial non-plaque tissue (intimal thickening). Tissue samples were homogenised and extracted consecutively for aqueous and organic extracts. Aqueous extracts were analysed using hydrophilic interaction chromatography (HILIC-)UHPLC-MS, whilst organic extracts by reversed-phase (RP-)UHPLC-MS. A panel of established as well as novel molecules, from several biological pathways, were identified as being dysregulated. These included free unesterified cholesterol (FUEC), oxidized cholesteryl esters, purines, pyrimidines, sphingolipids and acylcarnitines. A previously unassociated sphingolipid, namely phosphatidylethanolamine-ceramide (PE-Cer), was detected with high statistical significance ($p=9.8x10^{-12}$) and 2-fold reduction in plaque tissue. PE-Cer also demonstrated the highest (inverse) correlation to FUEC ($\rho=-0.76$). In pilot validation studies, human primary monocyte-derived macrophages (MDM) were treated with vehicle (naïve), acetylated-LDL (acLDL) (foam cells) and acLDL/FUEC (FUEC-loaded foam cells). The acLDL/FUEC-treated MDM demonstrated elevated apoptosis, and a 2-fold reduction in PE-Cer, in concordance with the findings in human tissue. This was accompanied by a reduction of SAMD8 RNA, the enzyme responsible for PE-Cer synthesis. Finally, a comprehensive examination of the sphingolipid pathway demonstrated an increase in de novo ceramide synthesis, further to the recognised in apoptosis hydrolysis of sphingomyelin (to ceramide). This provides insight for the role of PE-Cer and sphingolipids in advanced atherosclerosis and specifically in FUEC-loaded MDM foam cell apoptosis.

SESSION 13: LIPIDOMICS AND CARDIOVASCULAR DISEASE	Wednesday, June 26	10:15 a.m. – 12:00 p.m.

13D 11:20 a.m. – 11:40 a.m.	Plasma metabolomics profiles associated with endothelial health and dysfunction impose changes to endothelial glycan biosynthesis and reflect endothelial catecholamine response

PRESENTING AUTHOR: *Óttar Rolfsson, University of Iceland, Iceland*

CO-AUTHORS: *Sarah McGarrity, Hanne H. Henriksen, Per Johannsson*

Endothelial dysfunction (ED) contributes to diseases of the vasculature by influencing blood pressure, clotting and transport of fluids, nutrients and immune cells. Metabolic phenotypes associated with ED are not well characterised due to difficulties in assessing endothelial metabolism in situ. To address this, we recently built a cell scale metabolic network model of endothelial metabolism (iEC2812) and applied it to infer endothelial metabotypes from sepsis patient plasma metabolomics data. These highlighted changes to endothelial glycan metabolism. We subsequently hypothesized that endothelial glycocalyx maintenance contributes to endothelial dysfunction and updated our model (iEC2997) to more accurately account for endothelial glycocalyx synthesis and biomass. We then analysed, i) plasma metabolomics data from 20 trauma patients vs. 20 controls and ii) ASGR1del12 carriers vs. controls using iEC2997 to identify reactions associated with both dysfunctional and above normally healthy vasculature, respectively. Flux into the hexosamine biosynthetic pathway was altered in both cases. To verify this effect, we titrated HUVEC monolayers with physiological concentrations of catecholamines. Increased permeability and glycocalyx loss was verified by TEM, immunostaining and by permeability assays. UPLC-MS metabolomics analysis showed a drop in the intracellular concentrations of the glycan precursors UDP-glucose and N-acetyl-glucosamine. Extracellular measurements along with 13C-UL-glucose and 15N2-glutamine flux analysis supported lower turnover of glycocalyx intermediates and lower glycolytic and TCA cycle flux. In summary, metabolic network analysis of three independent plasma metabolomics datasets highlighted the importance of glycan synthesis to endothelial health. Induction of endothelial dysfunction in vitro is accompanied by compromised glycan synthesis.

13E 11:40 a.m. – 11:55 a.m.	Sphingolipidomics investigation of the ischemic brain injury

PRESENTING AUTHOR: *Ching-Hua Kuo, National Taiwan University, Taiwan*

CO-AUTHORS: *Hsi-Chun Chao, Tsung-Heng Lee, Sung-Chun Tang*

Stroke is among the three leading causes of death worldwide and is the most frequent cause of permanent disability. Significant changes of sphingolipid (SPL) levels have been observed after stroke. We conducted sphingolipidomic profiling of mouse brain tissue by liquid chromatography-electrospray ionization tandem mass spectrometry at 3 hour (hr) and 24 hr after 1 hr of middle cerebral artery occlusion (MCAO), and SPL were compared with those of the Sham control group. At 3 hr post MCAO, ceramides (Cers) exhibited increase in levels of long-chain Cers but decrease in very-long-chain Cers. Moreover, sphingosine, was decreased and S1P was increased at 3 hr after MCAO. Both long-chain and very-long-chain Cers showed an increased trend at 24 hr post MCAO. The administration of atorvastatin improved the neurological function of the mice and significantly reversed the SPL changes resulting from the ischemic injury. To further investigate the prognostic roles of SPL in patients with acute ischemic stroke, we collected plasma samples from acute ischemic stroke patients at 24 hr and 72 hr (n=90; prognosis: 40%) post stroke and non-stroke controls (n=64). The association study between the plasma ceramide concentrations and 3 months post stroke outcome revealed that the levels of long chain ceramides at 72 hr were significantly higher in patients with poor outcome. Long chain ceramides represented as potential prognostic markers for patients with acute ischemic stroke, and the network of SPL components that change upon ischemic damage may provide novel therapeutic targets for ischemic stroke.

SESSION 14: PLANT DEFENSE	Wednesday, June 26	10:15 a.m. – 12:00 p.m.

14A Session Keynote 10:15 a.m. – 10:45 a.m.	Elucidating insect resistance in tomato through genetical metabolomics

PRESENTING AUTHOR: *Ric de Vos, Bioscience, Wageningen Plant Research, Wageningen University and Research, Netherlands*

CO-AUTHORS: *Robert D. Hall, Roland Mumm, Roeland E. Voorrips, Ben Vosman*

Host plant pest resistance is becoming increasingly important in agriculture as more and more insecticides are being banned due to environmental concerns. In tomato, resistance towards several pests has been found in wild relatives (Solanum section Lycopersicon). To identify quantitative traits loci (QTLs) regulating pest resistance, we combined untargeted metabolomics using LC-PDA-Orbitrap FTMS with insect resistance assays, trichome phenotyping and genetic mapping. Screening a set of 14 accessions from close relatives of the cultivated tomato (S. lycopersicum, cv. Moneymaker) for resistance against different pest insects, including whitefly, thrips, caterpillar and aphids, we identified broad spectrum insect resistance in both S. galapagense and a few accessions of S. pimpinellifolium. An untargeted metabolomics comparison of leaves of resistant S. galapagense with those of its closest relative but sensitive S. cheesmaniae, containing and lacking type-IV trichomes respectively, indicated significantly higher levels of both a range of acylated sucroses and methylated flavonols in S. galapagense. Subsequent whitefly resistance screening and metabolomics comparison of a recombinant inbred line population and near-isogenic lines derived from a cross between S. lycopersicum and S. galapagense indicated a major QTL on chromosome 2 that controls the presence of both whitefly resistance, trichome types IV and V, and the accumulation of a specific set of phytochemicals including all identified acyl sugars and the methyl flavonols. We hypothesize that this QTL regulates the formation of glandular trichome type IV on the leaf epidermis, thus enabling the production and accumulation of the bioactive metabolites in this specific trichome type.

14B 10:45 a.m. – 11:05 a.m.	Dissecting the Genetic Basis of Variation in Tomato Fruit Metabolism and Pathogen Resistance through Multimodal Investigation of Wild Species Introgressions

PRESENTING AUTHOR: *Jedrzej Szymanski, Leibniz Institute of Plant Genetics and Crop Plant Research, Germany*

CO-AUTHORS: *Samuel Bocobza, Sayantan Panda, Prashant Sonawane, Pablo Cardenas, Justin Lashbrooke, Avinash Kamble, Nir Shachaf, Sagit Meir, Arnaud Bovy, Jules Beekwilder, Yury Tikunov, Irene Romero de la Fuente, Dani Zamir, Ilana Rogachev, Asaph Aharoni*

Wild tomato species represent a rich gene pool for numerous desirable traits lost during domestication. Here, we exploited an introgression population representing wild desert-adapted species and a domesticated cultivar to establish the genetic basis of gene expression and chemical variation accompanying the transfer of wild species-associated fruit traits. Transcriptome and metabolome analysis of 580 lines coupled to pathogen sensitivity assays resulted in the identification of genomic loci significantly linked with levels of hundreds of transcripts and metabolites. These associations occurred in functionally coherent hotspots that represent coordinated perturbation of metabolic pathways and ripening-related processes. We demonstrated the efficacy of our approach by discovering yet undisclosed components of the renowned Solanum alkaloids pathway and showing that fruit fungal resistance is associated with changes in the ripening regulatory network. Together, our results outline a framework for understanding metabolism and pathogen resistance underlying ripening and provide insights into key fruit quality traits.

14C 11:05 a.m. – 11:20 a.m.	Metabolomics application to unravel the biochemistry underlying enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria

PRESENTING AUTHOR: *Fidele Tugizimana, University of Johannesburg, South Africa*

CO-AUTHORS: *Lerato Nephali, Johan Huyser, Ian Dubery*

Drought conditions pose a growing threat to food security, affecting agronomically important crop plants. Although improved adaptation to drought has long been a pursuit of crop breeders, it has been difficult to achieve, since drought resistance is a quantitative trait controlled by multilayered cellular and molecular events. Hence, innovative and efficient strategies are imperatively required, such as the use of plant growth promoting rhizobacteria (PGPR). However, to devise these novel PGPR-based agricultural strategies, there is a necessity to firstly understand the physiology and biochemistry governing the interactions between beneficial bacteria and plants, and the resultant enhanced plant defences against abiotic stresses. Thus, we present the application of LC-MS-based metabolomics to elucidate the biochemical events and molecular mechanisms underlying PGPRs–maize interactions and the subsequent potentiation against drought conditions. The results of this study showed that the application of PGPRs induced alterations in different pathways of primary and secondary metabolism such as phenylpropanoid, flavonoid biosynthesis, fatty acid metabolism, amino acids and phytohormones pathways. Metabolic network analysis allowed the characterization of key metabolic 'hubs' that define the observed metabolic reprogramming. These changes were found to be related to enhanced drought resistance traits. Thus, our results suggest that selected PGPRs act as priming agents, enhancing plant resistance to abiotic stresses. Furthermore, the study contributes to ongoing efforts towards a comprehensive understanding of the PGPR-related defence priming phenomenon, which could play a significant role in alleviation of drought stress in plants, providing sustainable and economically favorable solutions to improve agricultural practices and crop productivity.

PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

SESSION 14: PLANT DEFENSE	Wednesday, June 26	10:15 a.m. – 12:00 p.m.

14D 11:20 a.m. – 11:40 a.m.	A strategy for the discovery and characterisation of health and decline biomarkers in British Oak trees using non-targeted metabolomics

PRESENTING AUTHOR: *Jasen Finch, Aberystwyth University, United Kingdom*

CO-AUTHORS: *Manfred Beckmann, Sandra Denman, John Draper*

Decline syndromes in British Oak trees are becoming increasingly important across the UK with the onset of climate change. These syndromes exhibit a multitude of complex disease phenotypes to which biotic and predisposing abiotic factors can contribute to over many growing seasons. A minimally destructive strategy for reproducibly sampling the metabolome content of living cell types in woody tissues has been developed that has allowed samples to be collected from trees at a number of sites across the UK. Trees have been extensively phenotyped for attributes reflecting aspects of decline status including; canopy condition, tree size and presence of biotic agents. Consideration of all of these phenotypic data has led to the development of a novel "tree health index", which describes the trees over a continuous decline spectrum. Sampled woody tissues have been analysed using untargeted flow infusion electrospray ionisation high-resolution mass spectrometry (FIE-HRMS), normal and reverse phase liquid chromatography high-resolution mass spectrometry (LC-HRMS) and gas chromatography-mass spectrometry (GC-MS) to provide comprehensive metabolome coverage. Using the machine learning algorithm random forest, metabolome features have been integrated with the tree health indexes revealing strong associations with a number of explanatory metabolites including amino acids involved in nitrogen metabolism/transport. These markers have been shown to be robust over 2 growing seasons and can adequately predict decline status in an independent set of trees. Identified markers have the potential to provide future diagnostic tools to monitor health status and even as a predictive tool for long term management strategies for oak decline.

14E 11:40 a.m. – 11:55 a.m.	Linking the volatilome and metabolome via plant defenses: stimulation of the salicylic acid pathway recruits natural enemies below ground

PRESENTING AUTHOR: *Camila Filgueiras, Cornell University, United States*

CO-AUTHORS: *Denis S Willett*

The metabolome and volitalome can be considered linked, overlapping sets. Traditionally, studies have looked at each in isolation with the implicit understanding that one influences the other. In plant-insect interactions this understanding is becoming more than implicit. Here we show that exogenous aboveground stimulation of the salicylic acid pathway induces release of volatiles by plant roots that recruit entomopathogenic nematode natural enemies belowground. This is one of the first examples of aboveground effects propagating belowground and points to a role of the salicylic acid pathway in mediating secondary plant defenses reliant upon volatile recruitment of natural enemies. Understanding the relationship between internal plant defense metabolites and volatiles released by plants that recruit natural enemies enhances not only our knowledge of the connection between metabolome and volitalome, but also our ability to control pests in the field.

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TECHNOLOGY

SESSION 15: DATA ANALYSIS & STATISTICS	Wednesday, June 26	10:15 a.m. – 12:00 p.m.

15A Session Keynote 10:15 a.m. – 10:45 a.m.	Is Metabolomics ready for the return of Artificial Neural Networks?

PRESENTING AUTHOR: ***David Broadhurst, Edith Cowan University, Australia***

Metabolomics data from in vitro and in vivo studies is more comprehensive and in depth than ever. Large data sets from metabolomics and flux studies are often daunting to analyze and interpret, especially when projects move quickly. A major focus of our lab has been in developing workflows and data analytics to allow rapid turnaround of data and fast iteration of biological experiments. Our goal is to quickly learn from one experiment, to design the next, and to iterate this process in a way that effectively impacts our projects, allowing us to identify novel biological mechanisms and pharmacodynamic biomarkers for our oncology and rare genetic disease programs. In this presentation, I will briefly discuss our multidisciplinary MS lab (metabolomics, lipidomics, fluxomics, and proteomics) and data systems. I will also present examples when metabolomics gave us unique insights into biological processes, including the development of a pharmacodynamic marker for a cholesterol pathway oncology target, an analysis of the tumor microenvironment across several models, and the modulation of metabolism in immunological models (in vitro and in vivo).

15B 10:45 a.m. – 11:05 a.m.	MS-DIAL 4.0: a computational workflow for ion mobility tandem mass spectrometry data in metabolomics

PRESENTING AUTHOR: ***Hiroshi Tsugawa, RIKEN, Japan***

CO-AUTHORS: ***Yoshifumi Mori, Yasuhiro Higashi, Aya Satoh, Sven Meyer, Kazuki Saito, Masanori Arita***

Ion mobility mass spectrometry provides the robust physiochemical measurement (or property) called collision cross section (CCS) of metabolites by increasing the peak capacities in mobility dimension. Although metabolite identification can be substantially improved in terms of accuracy and coverage, few programs can handle its raw data for peak picking, metabolite annotation, peak alignment, and statistical analysis. Here, we release a new software program, MS-DIAL 4.0, for data dependent MS/MS acquisition in liquid chromatography coupled with ion mobility tandem mass spectrometry (LC-IM-MS/MS). The program facilitates the metabolite annotation by integrating five-dimensional (5D) physicochemical properties, i.e., ion abundance, retention time (RT), CCS, m/z, and MS/MS spectrum. In addition, we developed the enriched mass spectral database for lipidomics containing the 5D properties for more than 200,000 molecules of 90 lipid classes. We showcase our workflow for plant lipidomics where the metabolic changes in heat stress are grasped by the 5D diagnostic criteria.

15C 11:05 a.m. – 11:20 a.m.	Chemically informed distance metrics for tandem mass spectrometry data

PRESENTING AUTHOR: ***Madeleine Ernst, Department of Congenital Disorders, Statens Serum Institut, Denmark***

CO-AUTHORS: ***Justin J.J. van der Hooft, Kyo Bin Kang, Asker Brejnrod, Louis-Félix Nothias, Ricardo R. da Silva, Pieter C. Dorrestein***

Untargeted mass spectrometry-based metabolomics has become a method of choice to evaluate differences and similarities in metabolite profiles in a multitude of applications and sample types. Pairwise dissimilarities across samples are often summarized using a distance metric followed by multivariate statistics such as principal coordinate analysis (PCoA) for comparative analyses. However, conventional distance metrics ignore the chemical structural relatedness between molecules. Thus, whilst two samples could contain chemically similar yet distinct molecules, they would be regarded as very different. Here, we propose two chemically informed distance metrics inspired by the UniFrac metric, a metric used to compare microbial communities by incorporating phylogenetic relatedness between observed organisms. Similar to microbes, molecules are also related to each other as they share biosynthetic pathways and consequently similar chemical substructures. We implement two versions of calculating chemical relatedness, one based on shared chemical class annotations, and one based on shared substructure patterns. Using datasets from diverse sources, we show how these metrics can improve multivariate statistics and subsequent data interpretation. For example, in the cosmopolitan plant family Rhamnaceae we observe high chemical dissimilarity across two phylogenetic clades producing chemically very distantly related compounds, such as flavonoids and terpenoids, a trend that could not be revealed using conventional methods. We expect that the proposed chemically-informed distance metrics will be of great benefit to any metabolomics fields studying complex mixtures.

SESSION 15: DATA ANALYSIS & STATISTICS	Wednesday, June 26	10:15 a.m. – 12:00 p.m.

15D 11:20 a.m. – 11:40 a.m.	Identifying biologically relevant modules in metabolomics and lipidomics data with Differential Network-based Enrichment Analysis (DNEA)

PRESENTING AUTHOR: *Alla Karnovsky, University of Michigan, United States*

CO-AUTHORS: *Gayatri Iyer, Janis Wigginton, William Duren, Marci Brandenburg, George Michailidis*

Metabolomics and lipidomics datasets are becoming increasingly large and complex, requiring powerful statistical and bioinformatics tools. A well-established approach to linking alterations in metabolite levels to specific biological processes is to map experimentally measured metabolites to known biochemical pathways and to identify the pathways that are significantly enriched with those. However, traditional enrichment analysis techniques have limited utility for the analysis of untargeted metabolomics and lipidomics data. We developed an alternative approach, which relies on extracting meaningful associations between metabolites/lipids directly from the experimental data. Our Differential Network Enrichment Analysis method (DNEA) uses joint structural sparsity estimation to build partial correlation networks from the data, performs consensus clustering to identify highly connected subnetworks, and uses Network-based Gene Set Analysis (NetGSA) to identify the differentially enriched subnetworks. We have extended the method, improving its versatility in situations such as unbalanced designs and low sample size relative to number of features. The program is implemented in Java as a client-server application with a GUI. The input to the program is a comma-separated file with metabolite measurements and the information about experimental groups. The output contains the list of enriched subnetworks ranked by significance and can be viewed as an HTML file, or exported into a network visualization software (e.g. Cytoscape). We tested DNEA in a number of publically available metabolomics and lipidomics datasets from a variety of diseases and will demonstrate that DNEA can identify alterations in both network structure and expression levels of interacting biomolecules that impact disease phenotypes.

15E 11:40 a.m. – 11:55 a.m.	Signature Mapping (SigMa): A new automatic tool for rapid processing of complex urine NMR spectra

PRESENTING AUTHOR: *Bekzod Khakimov, University of Copenhagen, Denmark*

CO-AUTHORS: *Bekzod Khakimov*, Nabiollah Mobaraki, Alessia Trimigno, Violetta Aru, Søren Balling Engelsen*

1D 1H NMR analysis of urine generates rich but complex data. Retrieving metabolite information from complex NMR spectra is a main bottleneck due to signal overlapping and chemical shift changes. This study illustrates a new method; Signature Mapping (SigMa), for identification and quantification of signature signals (SS) and allows rapid conversion of complex human urine NMR spectra into an informative metabolite table. SigMa compiles advanced multivariate data analysis algorithms for NMR data alignment, baseline correction, peak finding and peak deconvolution into user friendly software. The method utilizes an experimental data on unique chemical shift ranges of 166 SS representing 100 urine metabolites, enabling unambiguous metabolite identification in thousands of spectra simultaneously. In addition, an automatic peak finding option of SigMa allows detection of spectral regions (SR) of any unknown spin systems, and complex regions representing overlapped signals of several metabolites. This makes SigMa suitable for targeted and untargeted metabolomics. SigMa applies an individual MCR modelling for relative quantification of each SS, while complex SR are quantified using a binning approach. The performance of SigMa was evaluated using three NMR datasets and proved to be more efficient than the most often used binning approach. Processing of 40 spectra measured on urine of seven healthy individuals using SigMa showed an increase of the between individual variation while decreasing the within individual variation. SigMa also depicted the highest quantification accuracy and reproducibility of urine metabolites in large datasets containing several thousands of human urine NMR spectra.

NEW FRONTIERS

SESSION 16: SINGLE CELL	Wednesday, June 26	10:15 a.m. – 12:00 p.m.

16A Session Keynote 10:15 a.m. – 10:45 a.m.	Spatial metabolomics in tissues and single cells

PRESENTING AUTHOR: ***Theodore Alexandrov, EMBL, Germany***

Metabolites, lipids, and other small molecules exhibit complex and cell-specific spatial localization in tissues supporting tissue homeostasis in health and metabolism reprogramming in disease. Our team develops novel tools for spatial metabolomics to detect and interpret the roles of these molecules in tissues and single cells. I will present a recently developed cloud platform METASPACE for spatial metabolomics in tissues which provides a comprehensive community-populated resource for metabolism research and for spatial systems biology. I will also present a spatial single-cell metabolomics method which, by correlative in situ imaging of cell monolayers, provides for each cell its metabolic profile and assesses its optical, morphological, and fluorescent phenotype. These tools open novel avenues for understanding metabolism in tissues and on the single-cell level.

16B 10:45 a.m. – 11:05 a.m.	Comprehensive Single Cell Multi Omics

PRESENTING AUTHOR: ***Christian Berchtold, Institute for Chemistry and Bioanalytics, Switzerland***

CO-AUTHORS: ***Luca Rima, Stefan Arnold, Götz Schlotterbeck, Thomas Braun***

The comprehensive combination of various analytical methods is a key feature for the future of biochemical investigations. Analyzing metabolites and proteins from the same sample, especially from the same cell, might be extremely supportive of gaining a fundamental understanding of cellular processes. A recently developed single-cell picker [1, 2] allows the sampling and lysis of individual cells. This enables the picking of cells according to phenotypical appearance. In this proof of concept study, we used this system to deposit the lysate from one or a few cells on dedicated slides. Using a modified CAMAG TLC-MS interface, the cell lysate was transferred into a standard LC-MS system including chromatographic separation. Highly abundant metabolites such as glutamine or glutamic acid were detected to a level of single cells. Proteins remained on the microscope slide and were detected by antibody staining after LC-MS analysis of the same cell. Finally, we could demonstrate a strategy to combine several techniques to detect metabolites and proteins from the same sample. However, the concept needs further optimization according to sensitivity, robustness, and speed for quantitative high-throughput measurements.

16C 11:05 a.m. – 11:20 a.m.	Quantifying heterogeneity in drug uptake, metabolism and effect using Raman spectroscopy and mass spectrometry on the single-cell level

PRESENTING AUTHOR: ***Ahmed Ali, Leiden University, The Netherlands | RIKEN, Japan, Japan***

CO-AUTHORS: ***Yasmine Abouleila, Arno Germond, Mashaghi Tabari A, Thomas Hankemeier, Toshio Yanagida***

In the drug discovery field, monitoring drug uptake, its metabolism and response on the single-cell level has been extremely challenging. In this study, we show the potential of label-free single-cell analysis in drug discovery by utilizing a bi-modal analytical platform that combines Raman spectroscopy and mass spectrometry (MS). Raman spectroscopy was used to monitor the effect of tamoxifen on single-cells treated with the drug in a high-throughput and non-invasive manner. The same cells were then sampled and analyzed by mass spectrometry, in which, the concentration of the drug and its metabolite 4-hydroxy tamoxifen (4-OHT) were measured in each cell. MS measurements revealed a strong heterogeneity in the drug concentration across single-cells. This phenomenon was even more pronounced in the case of its metabolite. Despite this, the concentrations of both the drug and its metabolite correlated positively, confirming the drug uptake and drug metabolism in the cell. Moreover, Raman could not only detect the cellular metabolic changes caused by the drug, but also predict whether a given cell is affected by the drug or not with 97% accuracy using a PLS-DA model. Finally, we succeeded in correlating the drug effect (measured by Raman spectroscopy) and the concentration of the drug or its metabolite (measured by MS) on the single-cell level. The Raman peaks used in this correlation could be used as biomarkers for the drug activity in the cell. In conclusion, our integrated Raman-MS platform proved to be a powerful tool that could aid future drug discovery efforts.

SESSION 16: SINGLE CELL	Wednesday, June 26	10:15 a.m. – 12:00 p.m.

16D 4:35 p.m. – 4:50 p.m.	Relationships between cellular metabolite and drug uptake and transporter expression profiles

PRESENTING AUTHOR: *Marina Muelas, University of Liverpool, United Kingdom*

CO-AUTHORS: *Farah Mughal, Steve O'Hagan, Philip J. Day, Douglas B. Kell*

It is widely but erroneously believed that drugs get into cells by passing through the phospholipid bilayer portion of the plasma and other membranes. Much evidence shows, however, that this is not the case, and drugs cross biomembranes by hitchhiking on transporters for other natural molecules to which these drugs are structurally similar. We present untargeted metabolomics time course analyses of the uptake and secretion of metabolites in human serum by a number of human cell lines. We show how distinct the metabolic footprints of different cell lines are from one another. We subsequently compare the transcriptomes (by RT-qPCR or using published RNA-Seq datasets) and published proteomic expression profiles of cell lines with the uptake of substances (by LC-MS/MS). By employing mathematical methods we infer the variations in SLC and ABC membrane transporter expression to best explain the variation in metabolite uptake and the relationship to the function of the tissues from which these cell lines are derived. This analyses will also admit the production of quantitative structure-activity relationship (QSAR) models that will further aid in the prediction (and testing) of transporters responsible for the uptake and secretion a number of pharmaceutical drugs. We also utilise the Gini index (coefficient) as a novel means of characterising the variation in individual transporter distributions between these cell lines. Our results show that many transporters exhibit extremely high Gini coefficients, indicating a much higher degree of specialisation than is usually assumed.

16E 11:40 a.m. – 11:55 a.m.	A Single-Cell Look at Biological Nitrogen Fixation: Direct Determination of Metabolite Formulas from Isotopic Fine Structures in Heterogeneous Cell Populations

PRESENTING AUTHOR: *Tina Tran, The George Washington University, United States*

CO-AUTHORS: *Laith Z. Samarah, Rikkita Khattar, Sylwia A. Stopka, Christine A. Brantner, Paola Parlanti, Dušan Veličković, Jared B. Shaw, Beverly J. Agtuca, David W. Koppenaal, Christopher R. Anderton, Nikola Tolić, Gary Stacey, Ljiljana Paša-Tolić, Akos Vertes*

Many legumes, like soybean, do not depend on synthetic fertilizers for nitrogen uptake. Instead, they create mutualistic relationships with bacteria capable of biological nitrogen fixation. Specialized plant organs are created from these interactions (i.e., root nodules), were bacteria infected plant cells are intimately mixed with uninfected cells. Exploring the metabolic profiles for these heterogeneous systems requires single-cell analysis techniques. Here, we present the combination of fiber-based laser ablation electrospray ionization (f-LAESI), with 21 Tesla Fourier transform ion cyclotron resonance mass spectrometry (21T FTICR-MS) for the direct analysis of single cells. Direct determination of metabolite formulas from isotopic fine structures (IFS) was attained from our single cell data. Over 100 compounds were tentatively assigned based on ultra-high mass accuracy, and elemental formulas for 47 of these were verified by IFS. Comparing the calculated IFS patterns for possible metabolite ions with the experimental data enabled the identification of close-to-isobaric compounds with different elemental compositions. Infected cells showed higher abundances for nitrogen-containing compounds and lipids compared to uninfected cells. Compositional heterogeneity among infected cells was observed by determining the metabolic noise (η^2). Within a population of 124 infected cells, several primary metabolites essential for cellular growth and maintenance, and plant-specific secondary metabolites, exhibited relatively low η^2 ($\lesssim 0.50$). Conversely, lipids associated with plant and bacteroid membranes showed greater noise levels ($\eta^2 \geq 0.50$), indicating a larger variance in their amounts. Metabolic noise measurements can provide insight on how tightly the metabolite levels are regulated, and how that relates to their biological function.

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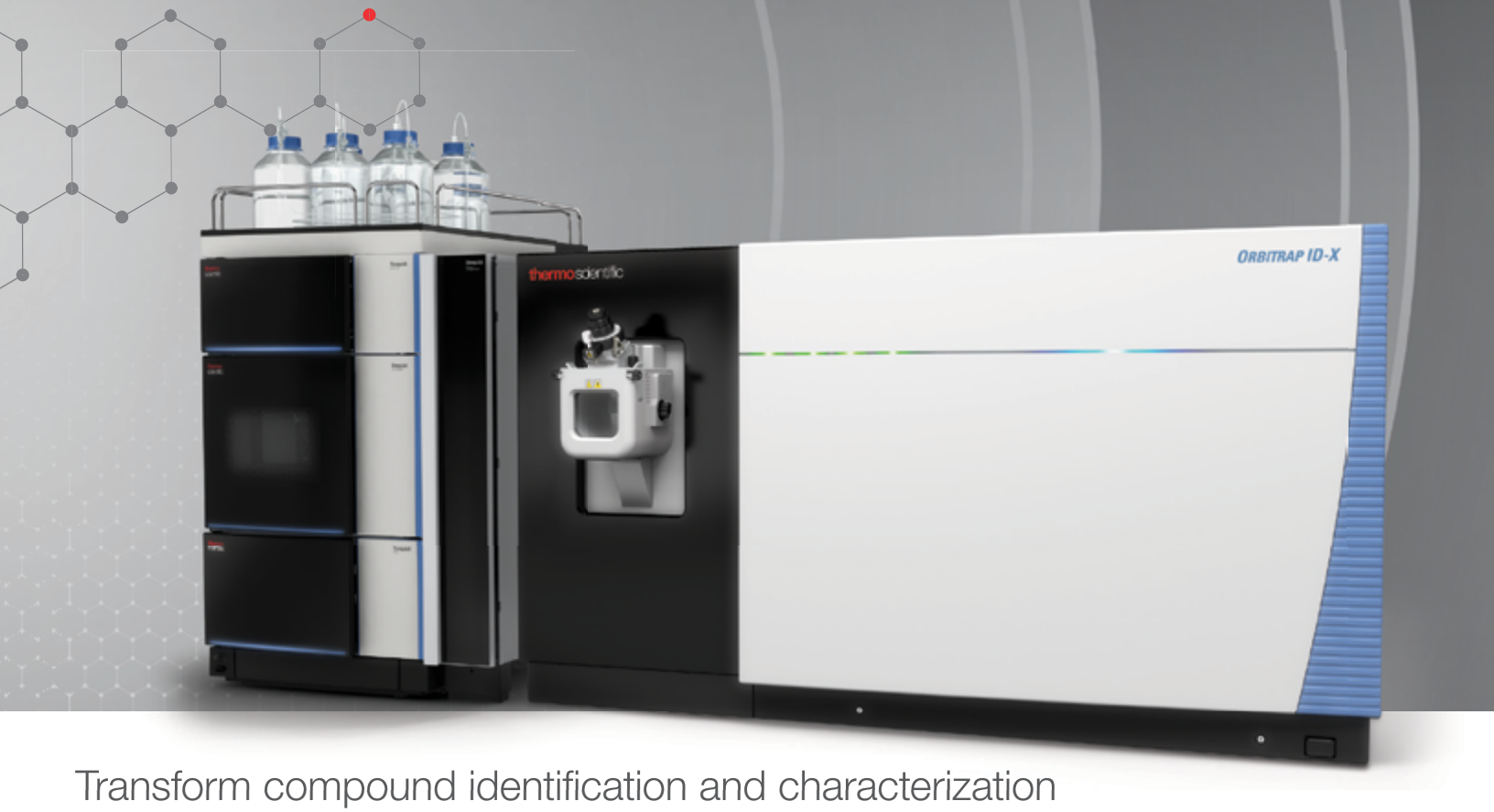

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SESSION 17: AGEING AND DISEASE	Wednesday, June 26	1:30 p.m. – 3:15 p.m.

17A Session Keynote 1:30 p.m. – 2:00 p.m.	Multiomic data integration using machine learning and data-driven inverse metabolic modelling - from diabetes to immune system modulation

PRESENTING AUTHOR: ***Wolfram Weckwerth, University of Vienna, Austria***

An integrated proteomics/phosphoproteomics/metabolomics platform is presented. This platform serves also as the basis for the Vienna Metabolomics Center (VIME) (https://metabolomics.univie.ac.at/). The data mining strategy is based on the integrative toolbox COVAIN (COVAriance INverse) for data processing, integration, multivariate statistical analysis, machine learning and data-drive inverse metabolic modelling [1]. We applied this integrative workflow to gestational diabetes mellitus (GDM) [2] and to the analysis of mTOR-dependent immune system modulation [3]. Activation of immune cells is accompanied by a metabolic reconfiguration of their cellular energy metabolism including shifts in glycolysis and mitochondrial respiration that critically regulate functional effector responses. However, while current mass spectrometry strategies identify overall or flux-dependent metabolite profiles of cells or tissues, they fail to comprehensively identify the checkpoint nodes and enzymes that are responsible for different metabolic outputs. Here, we demonstrate that a data-driven inverse modelling approach from mass spectrometry metabolomics data can be used to identify causal biochemical nodes that influence overall metabolic profiles and reactions. Using multiomics metabolomics, proteomics, phosphoproteomics, transcriptomics analysis as well as enzymatic activity measurements we identified metabolic signatures of energy signaling and macrophage differentiation. The presented concept of multiomics analysis, machine learning and data-driven inverse modelling allows for systematic integration of genome-scale metabolic reconstruction, prediction and analysis of causal biochemical regulation in microbes, plants, animals, human and their interactions.

17B 2:00 p.m. – 2:20 p.m.	Mass spectrometric analysis of sebum contents for classification of Parkinson's disease

PRESENTING AUTHOR: ***Drupad Trivedi, University of Manchester, United Kingdom***

CO-AUTHORS: ***Eleanor Sinclair, Depanjan Sarkar, Joy Milne, Monty Silverdale, Tilo Kunath, Roy Goodacre, Perdita Barran***

Parkinson's disease is a progressive neurodegenerative disorder that affects ageing population and around 1 in every 350 adults is diagnosed in the UK with Parkinson's disease (PD). Currently there are no definite diagnostic tests that can detect PD at early stages. This research is an effort towards early diagnosis of PD using biomarkers on skin, which anecdotally is known to smell differently in PD than in control. Sebum swabs from upper back of 276 participants consisting of PD on medication (n=139), drug naive PD (n=81) and control (n = 56) participants were collected across 28 different NHS sites in the UK. High-performance liquid chromatography was couple to time-of-flight mass spectrometry for separation and detection of metabolites extracted from sebum samples. After pre-processing, the resultant 700 features were carried forward for statistical analyses. Machine learning techniques consisting of support vector machines (SVM) and random forests (RF) were used for classification of data into two groups by using two subsets: (i) control and drug naive (ii) control and PD on medication. The metabolome measured was able to achieve classification accuracy by SVM and RF. Pathway enrichment analysis using all detected and identified metabolites showed significant enrichment ($p < 0.05$) of primary bile acid biosynthesis, sphingolipid metabolism, D-Arginine and D-ornithine metabolism as well as arginine and proline metabolism. Our results indicate a significant shift of endogenous metabolome in PD, causing an enhanced effect reflected on lipid-like molecules that are captured on the skin sebum.

17C 2:20 p.m. – 2:35 p.m.	Mechanistic model-driven exometabolomic characterisation of human dopaminergic neuronal metabolism

PRESENTING AUTHOR: ***Ronan Fleming, Leiden University, Netherlands***

CO-AUTHORS: ***Jennifer Modamio, Can Gulersonmez, Fatima L. Monteiro, Diana El Assal, Edinson L. Moreno, Miguel A.P. Oliveira, Cornelius C.W. Willacey, Laurent Heirendt, Zhi Zhang, Ben Cousins, Hulda S. Haraldsdóttir, Susanne Zach, Averina Nicolae, Siham Hachi, Amy Harms, Santosh Vempala, Bastian Hengerer, Jens C. Schwamborn, Ines Thiele, Enrico Glaab, Thomas Hankemeier, Ronan M.T. Fleming, German Andres Preciat Gonzalez***

Patient-derived cellular models are a powerful approach to study human disease, especially neurodegenerative diseases, such as Parkinson's Disease, where affected primary neurons, e.g., substantia nigra dopaminergic neurons, are almost inaccessible. Induced pluripotent stem cell-derived models of midbrain-specific dopaminergic neurons are increasingly used to investigate Parkinson's Disease. Starting with the comprehensive generic reconstruction of human metabolism, Recon3D, we generated the first constraint-based, genome-scale, in silico model of human dopaminergic neuronal metabolism (iNESC2DN). Transcriptomic data, obtained by RNA sequencing, and quantitative exometabolomic data, obtained by targeted mass spectrometry-based metabolomics were generated for in vitro neuroepithelial stem cell-derived cultures and supplemented by extensive manual curation of the literature on dopaminergic neurons. The predictions of the iNESC2DN model are consistent with neurobiochemical prior information and in concordance with measured fluxes of uptake and secretion of many extracellular metabolites by dopaminergic neurons in vitro. We leverage it to rank order the most important metabolite concentrations to quantify to maximally reduce the uncertainty associated with current predictions of normal dopaminergic neuronal metabolism in vitro, as well as optimally design experiments to measure metabolic perturbations associated with Parkinson's Disease. Finally, the iNESC2DN model provides a foundation for future targeted metabolomic and tracer-based metabolomic analyses of dopaminergic neurons. This illustrates the synergy between constraint-based computational modelling of metabolism and biology-driven quantitative bioanalytical chemistry.

SESSION 17: AGEING AND DISEASE	Wednesday, June 26	1:30 p.m. – 3:15 p.m.

17D 2:35 p.m. – 2:55 p.m.	Interleaving metabolic effects of sleep and aging

PRESENTING AUTHOR: *Arjun Sengupta, University of Pennsylvania, United States*

CO-AUTHORS: *Jennifer Choi Tudor, Joseph Baur, Ted Abel, Aalim Weljie*

Decreased sleep is a hallmark of modern society. Environmental factors such as stress as well as biological factors such as aging contributes to decreased sleep quantity and quality. Sleep deprivation (SD) has been linked to life threatening complications including learning and memory related neurodegenerative diseases and cardiometabolic disorders. The role of metabolic disruption in physiological effects of SD is emerging, but not completely understood. The nature of the interaction of SD with aging phenotypes is also unclear. Here we used a mouse model of 5 h acute sleep deprivation in young adult (2-4 months) and aged (20-22 months) mice to capture metabolic shifts as function of SD. Remarkably, numerous metabolites were perturbed post SD in young (24, 61, 53 metabolites in hippocampi, liver, and plasma, respectively) and aged animals (17, 42, 38 metabolites). Young animals demonstrated greater metabolic susceptibility post acute SD than aged animals. Specific SD metabolites in young animals (5, 19, 15 metabolites in hippocampi, plasma and liver, respectively) recapitulated a portion of the aging signature, thus demonstrating how SD makes the 'young seem old'. Hepatic signatures of ketosis were common to both groups, with greater prominence in the young animals. Altered hepatic NAD metabolism and urea cycle was also common signature of SD. Choline and acetylcholine pool in the young animals was specifically depleted in hippocampi, potentially linking metabolism to SD induced alteration in memory consolidation. These results form a foundation for understanding the systemic metabolic effects of sleep deprivation and aging.

17E 2:55 p.m. – 3:10 p.m.	Analysis of changes in the eye lens and aqueous humor under cataract development using quantitative metabolomics

PRESENTING AUTHOR: *Vadim Yanshole, ITC SB RAS / NSU, Russian Federation*

CO-AUTHORS: *Lyudmila Yanshole, Olga Snytnikova, Yuri Tsentalovich*

Introduction: The most common vision impairment of older people is caused by the formation of cataract (clouding of the eye lens). The protection of the lens is almost solely provided by metabolites; most of them are synthesized in the lens epithelium or enter the lens through the epithelial layer from the surrounding aqueous humor (AH). Therefore, changes in the metabolome of the lens and AH may help to establish the molecular mechanisms of the cataract onset. Methods: Quantitative metabolomic profiles of eye tissue extracts (lens, AH) were obtained with the combination of three methods – high-frequency 1H NMR and ion-pairing HPLC with optical (LC-OD) and high-resolution ESI-q-TOF MS detection (LC-MS) methods. Results: The concentrations of more than 80 metabolites were determined for four groups of samples: lenses and AH from cataract patients and lenses and AH from human cadavers. Conclusion: Our metabolomic data confirm the hypothesis that although the age-related cataract usually manifests itself as the opacification of the lens nucleus, the initial site of the cataract onset might be the lens epithelial layer. The most important for the lens protection metabolites – antioxidants, UV filters, osmolytes – are synthesized in the lens epithelial cells. The reduced levels of these metabolites were found in the cataractous lenses; that indicates that the cataract development may originate from the dysfunction of the lens epithelial cells. The increase in the concentrations in non-cataractous post-mortem tissues for other metabolites corresponds to the post-mortem processes. Acknowledgements: Supported by the RFBR (projects 18-33-20097, 18-34-00137, 17-03-00656).

PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

SESSION 18: FOOD APPLICATIONS 2	Wednesday, June 26	1:30 p.m. – 3:15 p.m.

18A Session Keynote 1:30 p.m. – 2:00 p.m.	Rapid Evaporative Ionisation Mass Spectrometry for detecting compounds related to consumer liking of meat

PRESENTING AUTHOR: ***Alastair Ross, AgResearch, New Zealand***

CO-AUTHORS: ***Paul Middlewood, Stefan Clerens, Patricia L Johnson, Arvind Subbaraj, Patrick Silcock, Graham T Eyres, Carolina E Realini***

Unbiased measurement of chemical components related to consumer liking is important for continuous improvement of breeding and meat processing for higher eating quality meat products, yet tools available for this purpose are limited. Rapid Evaporative Ionisation Mass Spectrometry (REIMS) is a mass spectrometry interface initially developed for surgery that allows real time analysis of samples, and is especially well suited for rapid analysis of meat. We hypothesised that within the metabolic signature of meat obtained using REIMS, it would be possible to detect compounds related to consumer liking. In a pilot study, 150 lambs were randomly selected within 10 different commercial mobs (n=15/group), and a 2 x 2 x 1 cm loin sample (Longissimus lumborum) collected for REIMS analysis. REIMS analysis was carried out in triplicate on 149 available samples in both positive and negative ionisation modes during two days. Associations between consumer panel (n=160 panellists) data and REIMS fingerprints were performed using PLS and OPLS-DA modelling, and confirmed using Pearson correlation (overall correlations) and ANOVA (between group differences). Meat with the highest overall liking scores was higher in compounds that are associated with aromatic amino acid biosynthesis and breakdown, while meat with lower overall liking scores was associated with a higher proportion of phospholipids. Using REIMS it was possible to detect compounds that were related to consumer liking of meat. In the future, this approach could be used to complement grading systems for identifying and predicting meat cuts with characteristics that maximize eating quality, and aiding consumer-driven breeding programmes.

18B 2:00 p.m. – 2:20 p.m.	A multi-platform metabolomics approach to predict sensory profiles of soy sauces in bouillon from key aroma and taste molecules

PRESENTING AUTHOR: ***Doris Jacobs, Unilever, Netherlands***

CO-AUTHORS: ***Ewoud van Velzen, Teun de Joode, Wilma du Chatinier, Ellen Evelingen, Sonja Kaal, Donny Merx, Herrald Steenbergen, Amy Harms, Thomas Hankemeier, Hye-Seong Lee, Frans-Jos Jansen***

Introduction: Soy sauces differ in their sensory perception due to variations in their ingredient compositions and brewing processes. The sensory quality of soy sauce-based foods can be improved by modulating key drivers for aroma and taste. However, the ensemble of molecules that affect flavour perception has not been completely identified. Method: In a design of experiment study, 10 different commercially available soy sauces added to chicken bouillon were varied in concentrations of the soy sauce, salt, and/or monosodium glutamate. In total, 25 specific and 5 holistic sensory attributes were evaluated by a trained panel. The molecular compositions were measured using NMR, element, GC-MS (volatile, non-volatile), LC-MS (global, amine, lipid) -based profiling. Partial least squares prediction models were built for each sensory attribute on a dataset of 118 molecules compiled from predictors that were initially selected from separate PLS analysis on each analytical platform. Results: Statistically significant PLS models were obtained for 19 sensory attributes such as fermented soybean flavour, chicken broth flavour and richness. Depending on the sensory attribute, different sets of known and unknown molecules were identified. For example, the model of richness included several organic acids, amino acids, dipeptides, pyrazines and aldehydes. In addition, several unknowns were identified that ranked even higher than some known kokumi peptides. Conclusion: With our multi-platform metabolomics approach we have now key flavour molecules at our control that finally will lead to improved sensory quality of soy sauce-based foods.

18C 2:20 p.m. – 2:35 p.m.	A metabolomic approach to the identification and validation of biomarkers of apple intake

PRESENTING AUTHOR: ***Aoife McNamara, Institute of Food and Health, Ireland***

CO-AUTHORS: ***Helena Gibbons, Diana Gonzalez-Pena, Breige McNulty, Lorraine Brennan***

The potential of individual food-intake biomarkers to objectively measure diet is a research area of interest. Improvements in dietary assessment are necessary to elucidate diet/health associations. The objective of this study was to identify novel biomarkers of apple intake and explore their potential to determine intake. Twenty volunteers consumed 360g of apples as part of an acute feeding study. Postprandial urine samples were collected at 2, 4 and 24 hours post-apple consumption for analysis. Subsequently a dose-response study was performed where volunteers consumed different portions of apples (50g, 100g or 300g) and fasting urine samples were collected for analysis. Xylose was quantified analysed on a 600-MHz Varian NMR spectrometer using the first increment of a NOESY pulse sequence. Dose-response data was used to develop calibration curves to determine apple intake in an independent free-living cohort (n=565) and used to classify individuals into categories of apple-intake. Multivariate analysis of NMR metabolomic data and time-series plots revealed that urinary xylose concentrations increased significantly at 4h post-apple consumption (2.19uM/osm), compared to the control food- broccoli (0.34uM/osm). In the dose-response study, urinary xylose concentrations demonstrated a linear increase as apple-intake increased ($r=0.406$; $p<0.0001$). Urinary xylose concentrations quantified in the free-living cohort (0.05 to 1.46mM) obtained strong agreement between biomarker-based intake classification and self-reported intake classification. Urinary xylose increased with increasing apple-intake. Xylose performed well as a ranking biomarker for grouping into apple-intake categories. Future work will combine xylose with other markers which may allow for determination of intake at an individual level.

SESSION 18: FOOD APPLICATIONS 2	Wednesday, June 26	1:30 p.m. – 3:15 p.m.

18D 2:35 p.m. – 2:55 p.m.	Brainfood metabolomics study (Icebreaker, Brave sub-study)

PRESENTING AUTHOR: *Kati Hanhineva, Afekta Technologies Ltd., Finland*

CO-AUTHORS: *Kati Hanhineva, Marika Laaksonen, Sanna-Maria Hongisto, Juhani Sibakov, Heli Diaz, Anton Mattsson, Ville Koistinen, Jussi Loponen*

Background: Cognitive performance is related to glucose metabolism and metabolic activation that are regulated by dietary factors. We studied the effects of brain-friendly diet (Brainfood) on metabolic and physiological parameters and cognitive performance in office workers at assumed metabolic risk (Brave study). Materials and methods: We conducted a diet-switch, 4-week intervention study on 84 volunteers with elevated plasma LDL levels in pre-screening. The Brainfood diet focused on regular meal frequency and optimal intake of polyunsaturated fats, fibre and salt, whereas the control diet was a typical western diet. Plasma samples were collected at the end of the lead-in, control and intervention periods, and analysed using liquid chromatography mass spectrometry (UPLC–QTOF-MS). Intervention efficacy was determined with a linear mixed model, and the fold changes of the metabolite levels were tested with t-tests corrected with Benjamini–Hochberg false discovery rate (FDR). Results: In total, we identified 37 differential metabolites (FDR-corrected $p < 0.05$), including acylcarnitines, amino acids, choline, 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid (CMPF), methylimidazoleacetic acid, monoacylglycerols, phospho¬lipids, piperine, and retinol. Several phosphatidylcholines and plasmalogens containing polyunsaturated fatty acids increased after the Brainfood diet, whereas lipids containing saturated or monounsaturated fatty acids decreased. The individual variability in metabolites was larger compared to the intervention effect. Conclusions: The Brainfood diet increased levels of CMPF and polyunsaturated fatty acid-containing phospholipids, likely originating from the intake of fatty fish. The reduced consumption of animal fats decreased their corresponding fatty acids in the lipid profile. Increased 3-methylhistidine was observed as a potential marker of poultry and plant-based protein intake.

18E 2:55 p.m. – 3:10 p.m.	Mass spectrometry based non-targeted metabolomics enables the identification of Amadori products in feces of formula-fed infants

PRESENTING AUTHOR: *Alesia Walker, Helmholtz Zentrum München, Research Unit Analytical BioGeochemistry, Germany*

CO-AUTHORS: *Nina Sillner, Daniel Hemmler, Monika Bazanella, Silke S. Heinzmann, Dirk Haller, Philippe Schmitt-Kopplin*

Non-targeted metabolomics can be used to identify food markers, without prior focusing on a set of small molecule ingredients or known food metabolites. Our interest was to investigate fecal metabolite profiles of breast- and formula-fed infants during the first year of life by LC-MS/MS and a subset by FT-ICR-MS. We observed that fecal samples of one month old infants, fed with whey-based formula milk, were dominated by four m/z values assigned as nitrogen containing compounds ($C_{18}H_{34}N_2O_8$, $C_{24}H_{44}N_2O_{13}$, $C_{12}H_{24}N_2O_7$ and $C_{18}H_{34}N_2O_{12}$), analyzed by FT-ICR-MS. HMBD annotation using m/z values resulted in one putative matching of fructosyllysine (FruLys), which is one of the most abundant Amadori products derived from food. Identification of four putative Amadori products was done with HILIC LC-MS/MS. Fragmentation experiments showed loss of a dipeptide for two features and multiple loss of water for all features, which is typical for glucose/ lactose moieties. The dipeptide fragment was assigned to leucylisoleucine (Leulle), which is present at the N-terminal sequence of the major whey protein β-lactoglobulin. The other two fragmented features showed typical lysine fragments. Based on the fragmentation, we identified two Leulle and two Lys Amadori products (FruLeulle, LactulosylLeulle, FruLys and LactulosylLys). Three of them were synthesized, fractionated and characterized by NMR, while FruLys was available as authentic chemical standard. All compounds were not detected in breastfed children and fecal levels of Amadori products decreased over time in formula-fed children due to solid food introduction. Thus, Leulle Amadori compounds could serve as food markers for consumption of whey-based formula milk.

SESSION 19: DATA INTEGRATION & DATA BASING 2	Wednesday, June 26	1:30 p.m. – 3:15 p.m.

19A Session Keynote 1:30 p.m. – 2:00 p.m.	The UniProt, Rhea, and SwissLipids knowledge resources for metabolomics and lipidomics

PRESENTING AUTHOR: ***Alan Bridge, Swiss-Prot group, SIB Swiss Institute of Bioinformatics, Switzerland***

CO-AUTHORS: ***Alan Bridge, Anne Morgat, Thierry Lombardot, Robin Liechti, Teresa Batista Neto, Sebastien Gehant, Parit Bansal, Jerven Bolleman, Kristian Axelsen, Lucila Aimo, Nevila Hyka-Nouspikel, Lou Götz, Dmitry Kuznetsov, Anne Gleizes, Anne Niknejad, Elisabeth Coudert, F Gisou van der Goot, Howard Riezman, Nicole Redaschi***

Our group specializes in the development and maintenance of expert curated knowledge resources for the life sciences. Here we describe recent work designed to improve the utility of these knowledge resources for integrated computational and experimental analyses of metabolic systems. Our starting point for this work is Rhea (www.rhea-db.org), a comprehensive expert-curated knowledgebase of over 11,500 biochemical reactions that uses the ChEBI (Chemical Entities of Biological Interest) ontology of small molecules to describe reaction participants, their chemical structures, and chemical transformations. We recently introduced Rhea as the standard for the annotation of enzymatic reactions in the UniProt Knowledgebase (UniProtKB, at www.uniprot.org). UniProtKB is a reference resource of protein sequences and functional annotation that currently includes over 150 million sequences and thousands of proteomes from all branches of the tree of life. The introduction of Rhea will significantly enhance the utility of UniProtKB as a basis to integrate and analyse metabolomic, proteomic and genomic data, to generate and annotate metabolic networks and models, to mine reaction data for pathway prediction and metabolic engineering, and to map metabolites to human diseases and associated genes and variants. We will describe selected tools and services that leverage Rhea and UniProt and practical examples of how to use them. We will conclude by describing how we use curated knowledge of lipid metabolic pathways in Rhea and cheminformatics approaches to build SwissLipids (www.swisslipids.org), a resource of over 500,000 known and theoretically possible lipid structures and biological knowledge that is fully mapped to ChEBI, Rhea and UniProt.

19B 2:00 p.m. – 2:20 p.m.	RIKEN Plant Metabolome MetaDatabase: an integrated plant metabolome data repository based on the semantic web

PRESENTING AUTHOR: ***Atsushi Fukushima, RIKEN Center for Sustainable Resource Science, Japan***

CO-AUTHORS: ***Mikiko Takahashi, Nozomu Sakurai, Toshiaki Tokimatsu, Hideki Nagasaki, Hideki Hirakawa, Takeshi Ara, Makoto Kobayashi, Miyako Kusano, Kazuki Saito, Masanori Arita, Norio Kobayashi***

Metabolome data provide new opportunities to gain a deeper understanding of plant metabolism relevant to both, plant and human health benefits. Major public repositories for general metabolome data have been launched over the past decade, but in terms of data sharing, many aspects remain unresolved especially for improving reanalysis, reusability and reproducibility. In this study, we developed the RIKEN Plant Metabolome MetaDatabase (RIKEN PMM, http://metabobank.riken.jp/), which stores gas chromatography-mass spectrometry-based (i.e. GC-MS-based) metabolite profiling data of plants together with their detailed experimental metadata, including sampling and experimental procedures. Our metadata are described using the Resource Description Framework (RDF) and standardised vocabularies, such as the Metabolomics Standardization Initiative Application Ontology (MSI-AO), to integrate with other life and biomedical science data on the World Wide Web. The RIKEN PMM implements intuitive and interactive operations for plant metabolome data, including raw data, mass spectra and metabolite annotations. The RIKEN PMM is suitable not only for scientists who are interested in metabolomic phenotypes but also for researchers who would like to investigate plant metabolomics approaches. Our framework can provide enough metadata required for reanalyzing and reusing the raw data, and will contribute to the development of yet another general-purpose metabolomics repository.

19C 2:20 p.m. – 2:35 p.m.	Contextualizing Metabolomics Data by Integrating Text Mining and Machine Learning

PRESENTING AUTHOR: ***Magnus Palmblad, Leiden University Medical Center, Netherlands***

The scientific literature contains a wealth of information for text mining and machine learning in the public domain, including Europe PMC with 35 million titles and abstracts, and 2.3 million open access papers. Much work has been done on extracting functional protein interactions and linking genes to diseases. Here I will describe how text mining chemical entities, with emphasis on metabolites, can be combined with machine learning and large-scale prediction of physicochemical properties to guide the selection of analytical methods in metabolomics, assess bias in metabolomics datasets, and place metabolomics data in wider contexts. The method is automated in scientific workflows, greatly facilitating its reuse and adaptation. Particular emphasis is placed on how to visualize the results from millions of named-entity recognitions in tens of thousands of articles in a single, informative, figure. The visualization styles may be new in the text mining but should be intuitive to practitioners of mass spectrometry-based metabolomics. Applications include drug development, data integration and business intelligence.

SESSION 19: DATA INTEGRATION & DATA BASING 2	Wednesday, June 26	1:30 p.m. – 3:15 p.m.

19D 2:35 p.m. – 2:55 p.m.	Metabolic Reaction Network-based Recursive Metabolite Annotation for Untargeted Metabolomics

PRESENTING AUTHOR: ***Zheng-Jiang Zhu, Chinese Academy of Sciences (CAS), China***

Large-scale metabolite annotation is a challenge in liquid chromatogram-mass spectrometry (LC-MS)-based untargeted metabolomics. Here, we develop a metabolic reaction network (MRN)-based recursive algorithm (MetDNA) that substantially expands metabolite annotations without the need for a comprehensive standard spectral library. Metabolites and their reaction-paired neighbor metabolites tend to share similar MS2 spectra due to structural similarity. Based on this rationale, MetDNA characterizes initial seed metabolites using a small tandem spectral library, and utilize their experimental MS2 spectra as surrogate spectra to annotate their reaction-paired neighbor metabolites which are subsequently served as the basis for recursive analysis. We further showcase the utility and versatility of MetDNA using different LC-MS instrumentations, data acquisition methods, and biological sample types, and demonstrate that about 2,000 metabolites can cumulatively be annotated from one experiment. MetDNA largely expands the annotation of metabolites, thereby allowing quantitative assessment for not just metabolic pathways but also multi-omic studies, such as integrative analysis between metabolomics and transcriptomics.

19E 2:55 p.m. – 3:10 p.m.	Sparse Multi-block PLS for Selection of Related Signals in Multi-platform Metabolomics Data

PRESENTING AUTHOR: ***Timothy Ebbels, Imperial College London, United Kingdom***

CO-AUTHORS: ***Kate Leary, Ibrahim Karaman, Goncalo Graca, David Herrington***

Combining data from multiple assays is of great value when attempting to identify unknowns in metabolomics. A key problem is how to associate signals from the same unknown in different assays. Statistical correlation can be helpful in finding associated signals, but is susceptible to noise, outliers and artefacts. Multivariate models are robust to these interferences and have proven their worth in many areas of metabolomic data modelling. Here, we evaluate sparse Multiblock PLS (sMBPLS) regression as an approach to integrate multiplatform data, with the objective of linking signals from the same molecule, to aid the process of identifying unknowns. We use real human serum NMR and LCMS data from a large cohort study with multiple epidemiological outcome variables. To evaluate the approach, we electronically spike signals at realistic levels into the real data. We find that signals from the same metabolite can be associated by this approach with a high degree of accuracy (AUROC=0.86 at 0.25 effect size, 10 spikes per block). We develop a novel visualisation tool allowing users to discover associated signals based on the sMBPLS model. Application to real outcome data shows that the method is able to associate signals from the same metabolites across multiple assays, indicating that this is a promising approach to aid identification of unknowns and discovery of biomarkers in multiplatform metabolomic data.

SESSION 20: REGULATORY SESSION

Translating metabolomics from academic science into regulatory practice: challenges and progress in pharmacology and toxicology

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SESSION 21: INFECTION AND IMMUNITY | Wednesday, June 26 | 3:45 p.m. – 5:30 p.m.

21A Session Keynote
3:45 p.m. – 4:15 p.m.

Understanding the metabolic perturbation in severe fever with thrombocytopenia syndrome

PRESENTING AUTHOR: ***Zeping Hu, Tsinghua University, China***

Severe fever with thrombocytopenia syndrome (SFTS) caused by a recently identified bunyavirus, SFTSV, is an emerging infectious disease with extensive geographical distribution and high mortality. Progressive viral replication and severe thrombocytopenia are key features of SFTSV infection and fatal outcome, whereas the underlying mechanisms are unknown. We revealed arginine deficiency in SFTS cases by performing metabolomics analysis on two independent patient cohorts, suggesting that arginine metabolism by nitric oxide synthase and arginase is a key pathway in SFTSV infection and consequential death. Arginine deficiency was associated with decreased intraplatelet nitric oxide (Plt-NO) concentration, platelet activation, and thrombocytopenia. An expansion of arginase-expressing granulocytic myeloid-derived suppressor cells was observed, which was related to T cell CD3-ζ chain down-regulation and virus clearance disturbance, implicating a role of arginase activity and arginine depletion in the impaired anti-SFTSV T cell function. Moreover, a comprehensive measurement of arginine bioavailability, global arginine bioavailability ratio, was shown to be a good prognostic marker for fatal prediction in early infection. A randomized controlled trial demonstrated that arginine administration was correlated with enhanced Plt-NO concentration, suppressed platelet activation, and elevated CD3-ζ chain expression and eventually associated with an accelerated virus clearance and thrombocytopenia recovery. Together, our findings revealed the arginine catabolism pathway–associated regulation of platelet homeostasis and T cell dysregulation after SFTSV infection, which not only provided a functional mechanism underlying SFTS pathogenesis but also offered an alternative therapy choice for SFTS.

21B 4:15 p.m. – 4:35 p.m.

Hijacking of host cellular function by opportunistic pathogens during intracellular infections

PRESENTING AUTHOR: ***Volker Behrends, University of Roehampton, United Kingdom***

CO-AUTHORS: ***Natalia Bravo-Santano, James K Ellis, Luis M Mateos, Yolanda Calle, Hector C Keun, Michal Letek***

Staphylococcus aureus is one of the leading causes of hospital-acquired infections and a major human pathogen worldwide. As a facultative intracellular pathogen, S. aureus is able to invade both phagocytic and non-phagocytic cells. The emergence of multidrug-resistant strains, such as methicillin-resistant S. aureus (MRSA) has made treating S. aureus infections significantly more complicated. Therefore, there is an urgent need to find novel therapies. This study aims to identify and target host molecular pathways exploited by MRSA during intracellular infection with a view of identifying possible host-directed therapies. We employed stable-isotope labelling coupled to GC/MS and NMR-based metabolic profiling to investigate the impact of bacterial infection on host carbon metabolism. We found extensive metabolic re-routing (a block of mitochondrial uptake of pyruvate resulting in a decreased flux through the TCA cycle from glucose) and detected several distinct metabolic changes that suggested starvation-induced autophagy in infected cells. These changes included increased uptake but lower intracellular levels of glucose, low abundance of several essential amino acids as well as markedly upregulated glutaminolysis. We identified the molecular cascade activated in our infection model and showed that most of the autophagosomes detected in infected cells did not contain bacteria, suggesting that S. aureus induces the autophagic flux during cell invasion for energy generation and nutrient scavenging. Accordingly, drug-induced inhibition of AMPK, the responsible host kinase, hampered S. aureus intracellular proliferation. Our findings show that targeting host pathways essential for MRSA survival is a viable therapeutic strategy against this versatile pathogen.

21C 4:35 p.m. – 4:50 p.m.

Diagnosis of Aspergillosis based on metabolomics data – application of relative expression analysis-RXA to minimize interindividual metabolic differences

PRESENTING AUTHOR: ***Joanna Godzien, Medical University of Bialystok, Clinical Research Centre, Poland***

CO-AUTHORS: ***Joanna Godzien, Marcin Czajkowski, David Rojo-Blanco, Cristina Cunha, Katrien Lagrou, Johan A. Maertens, Coral Barbas, Agostinho Carvalho***

Invasive aspergillosis (IA) is an infection that affects immunocompromised hosts. Due to the increasing occurrence and despite available antifungal therapy, IA is a leading cause of mortality in the ICU. To asses this complex problem a multiplatform metabolomics approach was implemented. Serum and bronchoalveolar lavage fluid samples from immunosuppressed patients, with and without IA, were profiled across three analytical platforms including LC/MS, GC/MS, and CE/MS. Due to the large intra-group variation caused by the variety of medical complications, apart from canonical chemometrics, a novel approach was applied. Exploitation of the data was performed using the Top Inter-GEne Relation-(TIGER) machine learning classifier, which focuses on the relative order of features rather than their raw values. The specialized evolutionary algorithm extends the concept of Relative Expression Analysis-(RXA) and enables the search for complex interactions and patterns in the metabolomics data. In this way, we found several interesting metabolic classifiers. As an example: if the concentration of glutamine is higher than the concentration of amino-caprylic, then with 80% probability such sample can be classified as a case. Moreover, changes between native forms of metabolites and their epi-forms were evaluated, revealing for example significance of methylation processes over acetylation. Extensive data analysis of two types of biological samples and multiplatform data integration allowed to determine metabolic differences between infected and uninfected patients. This leads us to define the metabolic signature of the predisposition to the IA. This metabolic signature was supported by clinical parameters to characterize the profile of such patients better.

SESSION 21: INFECTION AND IMMUNITY	Wednesday, June 26	3:45 p.m. – 5:30 p.m.

21D 4:50 p.m. – 5:10 p.m.	Alterations of systematic metabolism upon viral infection: from metabolite profiling to isotope tracing

PRESENTING AUTHOR: ***Kristaps Klavins, CeMM - Research Center for Molecular Medicine, Austria***

CO-AUTHORS: ***Alexander Lercher, Bettina Gürtl, Andreas Bergthaler***

Viral infections cause severe perturbations of a host metabolome that goes along with inflammation, tissue damage, and metabolic reprogramming. In this study, we investigated changes in systematic metabolism caused by chronic viral infection. We infected mice with lymphocytic choriomeningitis virus which is a well-established and pathophysiologically relevant model for chronic viral infection. We performed targeted quantitative profiling of more than 180 serum metabolites, including amino acids, biogenic amines, acylcarnitines, sphingolipids and glycerophospholipids from infected C57BL/6J wildtype (WT) mice, by LC-MS/MS. We observed wide-spread metabolic alterations and, most notably, metabolites of the urea cycle were significantly changed. The urea cycle is a central metabolic pathway expressed in the liver. To fully characterize infection-induced changes of this pathway, we established a LC-MS based method for quantification of all urea cycle metabolites and the closely connected tricarboxylic acid cycle. This method allowed us to confirm previous results as well as provided evidence of infection-induced reprogramming of the urea cycle. To validate these findings we performed an in vivo tracing experiment using 13C labeled metabolites. For reliable isotopologue detection, we employed high-resolution mass spectrometry and identified significant changes in degradation and synthesis rates of metabolites. Employing and integrating complementary metabolomics approaches led to the identification of virus-induced reprogramming of the urea cycle and altered systemic metabolite levels during chronic viral infection.

21E 5:10 p.m. – 5:25 p.m.	Characterisation of the human milk lipidome using liquid chromatography-ion mobility spectroscopy-mass spectrometry reveals novel lipids

PRESENTING AUTHOR: ***Alexandra George, The University of Western Australia, School of Molecular Sciences, Australia***

CO-AUTHORS: ***Melvin Gay, Donna Geddes, Kevin Murray, Robert Trengove***

The human milk (HM) lipidome is complex, with inter- and intra-individual variation present during the day, throughout a feed, and throughout lactation. These lipids provide over 50% of the daily infant energy requirements, and are known to contribute to immune cell metabolism and activation, and neural and retinal development. Although years of HM research have investigated the total fatty acid composition, these fatty acids are predominantly packaged as triacylglycerides in fat globules. The positioning of the fatty acids on the triacylglyceride is critical for infant absorption, therefore analysis of individual fatty acids does not accurately represent the HM lipidome and its physiological relevance to the infant. Liquid chromatography–ion mobility spectroscopy–mass spectrometry (LC-IMS-MS) methodology has demonstrated its ability to more comprehensively characterize triacylglycerides in bovine milk, but has not been applied to HM. To characterise the HM lipid profile, untargeted LC-IMS-MS was carried out on HM samples from a longitudinal cohort. Over 200 triacylglycerides and their fatty acid arrangements were identified, many of which have not previously been identified in HM. The abundance of each triacylglyceride, such as C61H106O6 (22:0/16:0/20:4), was lower in pre-feed samples and higher in post-feed samples, and typically increased throughout the day. C61H106O6 has potent antimicrobial properties and was measured in significantly lower concentrations in the milk of mothers with infants who more frequently suffered infections. The additional orthogonal separation offered by ion mobility can more accurately profile HM lipids and provides an opportunity to fully characterise the HM lipidome and its impact on infant outcomes.

PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

SESSION 22: ENVIRONMENT & TOXICOLOGY	Wednesday, June 26	3:45 p.m. – 5:30 p.m.

22A Session Keynote 3:45 p.m. – 4:15 p.m.	Metabolomics and toxicity studies of zebrafish embryos exposed to environmental contaminants

PRESENTING AUTHOR: ***Pim Leonards, Vrije Universiteit, Netherlands***

CO-AUTHORS: ***Jessica Legradi***

Per- and polyfluorinated alkylated substances (PFASs) have been widely used as organic surfactants in industrial and consumer products. While these applications made PFASs very appealing their properties make PFAS very persistent in the environment. In this study we performed a chemical alternatives assessment using metabolomics between PFOA and PFOS and alternative chemicals to be able to select environmentally friendly compounds. Zebrafish embryos (Danio rerio) were chronically and acutely exposured (0.1 µM , 1 µM , 10 µM) to PFOA and PFOs and its alternatives PFHxS, PFBS, siloxanes (D4, D5 and TMS), and GenX to study the mechanism of toxicity. No significant effects on mortality, visual developmental abnormalities, and swimming activity were observed for all compounds. Embryos were subjected to further investigation in an attempt to address potential genetic and metabolomic effects.Transcription of the DNA damage gene was significantly up-regulated by over 2-fold relative to the control vs. PFOA, PFHxS, PFOS and Genx. Interestingly, the lipid metabolism gene was up-regulated by over 2-fold in the embryos exposed to PFOS and GenX. These observations corresponds well with the metabolomics data that showed the regulation of many lipids (PC, PE, TAG, SM). Based on the results of this study, alterations in lipid metabolism (glycerophospholipids) may be a metabolic pathway affected by both PFASs and their alternatives. GENX, as alternative to PFOA, displayed a pattern of similar activity as the PFASs. The alternatives D4, D5 and TMS appears to have a lower impact on metabolic pathways (e.g. lipids) than PFOA.

22B 4:15 p.m. – 4:35 p.m.	Gestational and post-natal exposure to ambient air pollution: a metabolomics approach

PRESENTING AUTHOR: ***Marina Tavares, University of Sao Paulo, Brazil***

CO-AUTHORS: ***Andréa T. Faccio, Aline Klassen, Amanda V.B. das Dores, Daniel R. Oliveira, Lucas H.F. da Silva, Mariana M. Veras, Paulo Saldiva, Massuo J. Kato, Ernani Pinto Jr., Joao P.S. Farah***

According to WHO (2016), 91% of people living in cities are exposed to air pollution in levels above the recommended limits for air quality. Ambient air pollution has been associated with a number of acute and chronic conditions, including obstructive pulmonary disease, ischaemic heart disease, and lung cancer. Therefore, there is a pressing interest in understanding the underlying metabolic mechanisms by which atmospheric air pollution affects human health. An untargeted metabolomics approach was envisioned here to evaluate the effects of pre- and post-natal exposure to air pollution. A cohort of 24 pregnant mice was exposed during pregnancy to either filtered air or PM2.5 enriched polluted air. Offspring were further exposed: filtered air (n=35), and polluted air (n=39). Urine samples were collected and analyzed by HILIC-MS (zwitterionic-sulfobetaine column). Raw data were preprocessed (XCMS) and statistically evaluated using uni- and multi-variate approaches (SIMCA-P). Group classification clearly indicated the occurrence of metabolic alterations in mice as a result of both maternal and offspring exposure. For the mothers, 108 metabolites were putatively identified out of 137 discriminant metabolites and many chemical classes were impacted such as peptides, fatty acids, amines, and ketones. For the offspring, several group comparisons were possible, resulting in alterations of metabolic pathways associated to histamine and tryptophan biochemistry. A deeper evaluation of the metabolic pathways revealed by this work can greatly contribute to understand the link between air pollution and disease development, and perhaps, lead to the setting of new regulations and public health initiatives.

22C 4:35 p.m. – 4:50 p.m.	Association between coffee consumption, plasma metabolites and hypertension risk

PRESENTING AUTHOR: ***Wei Jie Seow, National University of Singapore, Singapore***

CO-AUTHORS: ***Wei Jie Seow, Wenchi Pan, Deron R. Herr, Federico Torta, Chin Meng Khoo, Markus R Wenk, Tai E Shyong, Rob M. van Dam***

Background: Coffee is one of the most popular beverages in the world. It is known that coffee drinking is associated with short-term spikes in blood pressure. However, the associations between coffee consumption, blood metabolites and hypertension risk in Asian populations are still largely unknown. Objectives: To assess the associations between dietary coffee consumption and hypertension risk, with potential mediation via blood metabolites. Methods: We used data from 3,020 participants of the population-based Singapore Prospective Study Programme (SP2) cohort. Amino acids, acylcarnitines and lipids in pre-diagnostic blood were measured using mass spectrometry. Logistic regression models were used to estimate the odds ratios (ORs) and 95% confidence intervals (CIs) for the association between coffee, metabolites and hypertension risk, adjusting for potential confounders. Mediation analysis was used to estimate the proportion of mediated effect via the metabolites on the association between coffee consumption and hypertension risk. False discovery rate (FDR) was evaluated using the Benjamini-Hochberg method. Results: Coffee consumption was associated with a 13% higher risk of incident hypertension for each cup of coffee consumed per day (OR=1.13,95%CI=1.02-1.26,P-value=0.024). Coffee was also associated with significant changes in the levels of 59 metabolites (FDR<10%), including sphingolipids and acylcarnitines. Levels of three metabolites (Cer d18:1/22:0,SM d16:1/24:0,HexCer d16:1/22:0) were directly associated with hypertension risk. Part of the putative effect of coffee intake on increasing hypertension risk was estimated to be mediated via these metabolites. Conclusions: Higher coffee consumption was found to be associated with higher hypertension risk, with possible partial mediation through blood ceramides and sphingomyelin concentration.

SESSION 22: ENVIRONMENT & TOXICOLOGY	Wednesday, June 26	3:45 p.m. – 5:30 p.m.

22D 4:50 p.m. – 5:10 p.m.	Small molecules and lipids preserved over thousands of years in mummified humans

PRESENTING AUTHOR: ***Kevin Quinn, University of Colorado Anschutz, United States***

CO-AUTHORS: ***Charmion Cruickshank-Quinn, Spencer Mahaffey, Roger Powell, Richard Reisdorph, Nichole Reisdorph***

Background: The mummified remains of ancient persons can provide clues about their environment, health, and death. While previous studies have focused on genetic and proteomic analyses, few studies have utilized untargeted metabolomics for either exploratory purposes or to gain insight into the mummification process. The goal was to determine differences associated with age and mummification process. Methods: The tissues from four, naturally preserved mummies were examined: Oetzi the Iceman (5,300 years old), Scythian Warrior (2,400 years old), Scythian Princess (2,400 years old), and a mummy found in an apartment in Vienna, Austria (4 years old). Samples were prepared using solid phase extraction to obtain the following four fractions: aqueous small molecules, fatty acids, neutral lipids, and phospholipids. Fractions were analyzed using liquid chromatography mass spectrometry (LC-MS) in positive and negative ionization mode with electrospray time-of-flight MS. Results: There were between 2,400 and 3,070 compounds detected in the mummified tissue samples. These compounds included oxidative stress-related compounds and bacterial/microbiome-associated metabolites. The lipid peroxidation product, 4-oxo-nonenal was detected in all the mummified samples, while urobilin (a degradation product of heme), and the antioxidant uric acid were only detected in the apartment mummy. The lipid PI(40:3) showed a pronounced decrease with time based on the age of the mummy that became undetectable at 5,300 years. The top enriched pathways (FDR < 0.0001) were sphingolipid metabolism and glycerophospholipid metabolism. Conclusion: Results suggest that certain small molecules and lipids can survive for millennia following the mummification process and that oxidation could indicate length of mummification.

22E 5:10 p.m. – 5:25 p.m.	Food preservative zinc oxide nanoparticles (ZnONPs) induces neurobehavioral deficits in mice with altered gut microbiome and dysregulated serum and hippocampus metabolome

PRESENTING AUTHOR: ***Chang Chen, Chongqing Medical University, China***

CO-AUTHORS: ***Xuejun Jiang, Xia Qin, Shanshan Zhang, Yujia Zhang, Lulu Bai, Tianxiong Wang, Zhen Zou, Chengzhi Chen***

Zinc oxide nanoparticles (ZnONPs) is widely used as food preservative, but its impact on health has not been fully elucidated. We studied the effect of orally administrated ZnONPs on the neurobehavior of mice, as well as the possible mechanism from the perspective of gut microbiome and serum and hippocampus metabolome. Results show that mice administrated with ZnONPs (n = 8) at equivalent dose to human food exhibited lower numbers of cross platform, longer escape latency in Morris water maze, and shorter total distance in open field test than their counterparts (n = 8). Eight bacterial clades were downregulated, while seven upregulated in the gut of mice in ZnONPs group. Twenty-six differential metabolites were found from serum and four from hippocampus. Correlation analysis found that the differential bacterial clades had a close relationship with the differential metabolites. We conclude that oral administration of ZnONPs may cause neurobehavioral deficits in mice, which is associated with a crosstalk between altered gut microbiome and serum/hippocampus metabolome.

SESSION 23: NEW INSTRUMENTATION	Wednesday, June 26	3:45 p.m. – 5:30 p.m.

23A Session Keynote 3:45 p.m. – 4:15 p.m.	Integration of MS imaging and LC-MS techniques – discovering tissue heterogeneity with the accuracy and depth of traditional profiling tools

PRESENTING AUTHOR: ***Matthew Lewis, Imperial College London, United Kingdom***

LC-MS, GC-MS and NMR spectroscopy are generally considered to be the core analytical approaches for metabolic profiling, however they are far from optimally applied in a number of cases including the analysis of biological tissues and high throughput analysis. At Imperial College London, capitalizing on the depth of experience within our National Phenome Centre and mass spectrometry imaging (MSI) facilities, we are advancing the integration of metabolic profiling approaches in an effort to realise the broader potential of technology-driven translational medicine. Although metabolic imaging by mass spectrometry is a sensible approach for the analysis of highly heterogeneous tissues, the approach suffers from poor sensitivity and the lack of ion structure information such as MS/MS or collisional cross section. Combining MSI with LC-MS can potentially solve these problems and also serve as a core for the integration of clinical chemistry and histopathology. Our approach utilizes serial sections, where one section is subjected to metabolic imaging by desorption electrospray ionization (DESI). The MSI data is analysed by supervised multivariate methods to identify histologically homogeneous segments and then the tumour part is segmented further using an unsupervised approach. The positions of discrete sub-histological phenotypic segments are registered on the following sections and dissected using laser capture microdissection. The resulting samples are extracted and subjected to LC-MS-based metabolic profiling. This approach allows us to perform traditional metabolite identification, spatially resolved quantification and detection of low-abundance species in tissues. Furthermore, the approach also allows the broader integration of other systems biology tools (genomics, transcriptomics, proteomics) with histology.

23B 4:15 p.m. – 4:35 p.m.	Mapping Hydrophilic Intermediates of the Central Carbon and Energy Metabolism (CCEM) by Ion Pairing and Ion Exchange Chromatography – Tandem MS

PRESENTING AUTHOR: ***Gerd Balcke, Leibniz-Institute of Plant Biochemistry, Germany***

CO-AUTHORS: ***Mohamad Saoud, Ludger Wessjohann, Alain Tissier***

Metabolomic analysis of hydrophilic metabolites is required in many research areas such as cancer biology, infection studies, diabetes, plant biology and in the attempt to understand basic principles of cellular regulation. However, quantitative and reproducible analysis of charged and hydrophilic CCEM intermediates is a challenging task which requires specific quenching/ extraction methods along with protection against degradation and inter-metabolite conversion. Using a combinatorial approach of ion pairing and ion exchange LC-MS/MS we separated and detected 152 phosphorylated, zwitterionic and carboxylated hydrophilic metabolites. We assessed cold quenching and cryo-extractions under various pH conditions and categorized stability conditions for metabolite classes such as nucleotides, nucleosides, CoA esters, TCA acids, sugar bisphosphates, etc., in different cell extracts. We demonstrate that under specific pH conditions even very labile metabolites such as CoA esters, nucleotides, sugar bisphosphates, GSH or NAD(P)(H) cofactors can be stabilized over 24 h at 4 °C. By spiking various non-labeled metabolites to U13C-labelled E.coli cells immediately before cold quenching/extraction we quantified the turnover and interconversion rates of various metabolites and pinpoint minimum bias conditions for the analysis of small molecule intermediates of the CCEM. We provide full solutions to assess CCEM intermediates of prokaryotes (in suspension), cancer cells (adherent) and snap-frozen plant tissue.

23C 4:35 p.m. – 4:50 p.m.	High-Resolution µ-scaled Magic-Angle Spinning NMR Mapping of diseased rat brains

PRESENTING AUTHOR: ***Covadonga Lucas-Torres, CEA Saclay, France***

CO-AUTHORS: ***Yusuke Nishiyama, Alan Wong***

In vivo MRS is a powerful spectroscopic technique for unveiling the metabolic distribution in tissue lesions. However, it can be limited by the spectral and spatial resolution for a comprehensive and unbiased metabolomic investigation. Localized NMR-based metabolic profiling on excised tumorous rat brain has been accomplished herein for the first-time under the recently introduced HR-µMAS technology (1). A total of 108 biopsies have been systematically sampled from 4 specific brain slices (2 control and 2 tumor) with an excellent spectral resolution (6 ppb at 11.7 T) and spatial sampling resolution (a voxel of 0.6 mm3). These resolutions are superior to those acquired from today's advanced localized in vivo MRS (with MRI). The excellent spectral data quality has allowed 95% of the acquired data for generating reliable statistical models. The initial multivariate data analysis discriminates between control and tumor, allowing for a preliminary inspection of the data and identification of the principal metabolic profile of the tumor (i.e. increase in cholines, taurine and glucose; decrease in NAA). The superb resolution has also unbiasedly unfolded the increased level of glycine content, in which the signal in in vivo MRS is often overlapped with that of myo-inositol. Moreover, the study has also capitalized the good spatial sampling resolution and revealed the different metabolic distributions across the brain slice. This study with HR-µMAS NMR offers a glimpse of its potential for investigating µg-scaled tissue biopsy.

SESSION 23: NEW INSTRUMENTATION	Wednesday, June 26	3:45 p.m. – 5:30 p.m.

23D 4:50 p.m. – 5:10 p.m.	Electroextraction-based sample preparation strategies for enrichment of low-abundant metabolites

PRESENTING AUTHOR: ***Peter Lindenburg, Analytical Biosciences & Metabolomics, Leiden Centre for Applied Bioscience, Leiden University, Netherlands***

CO-AUTHORS: ***Amar Oedit, Yupeng He, Thomas Hankemeier***

In biomass-limited samples, such as for example mouse brain tissue samples, the analysis of low-abundant metabolites remains a challenge as these metabolites fall below the limit of detection of these techniques. SPE- and LLE-based sample pretreatment procedures suffer from practical limitations (i.e. sample handling) when analyzing biomass-limited samples. Electroextraction (EE) is an electromigration-based sample pretreatment technique which depends on the application of an electric field over a liquid-liquid system that consists of immiscible organic donor and aqueous acceptor phases. The discontinuous electric field that is present in such liquid-liquid systems is the driving force behind the analyte enrichment capacity of EE. As a consequence, EE-based sample pretreatment improves the detection limits and enables analysis of low-abundant metabolites and is therefore highly suited for miniaturized, high-throughput metabolite analysis. Furthermore, it allows for efficient sample handling as well as pretreatment. EE specifically targets ionic analytes and is therefore very suited for analysis of the metabolome, which comprises ±80% of charged/chargeable metabolite species. Moreover, EE offers excellent possibilities for online integration in analytical platforms. This lecture presents analysis of low-abundant metabolites with several analytical platforms in which EE is incorporated, i.e. continuous-flow micro-EE, three-phase EE and hyphenated EE techniques (EE-DI-MS, EE-LC-MS and EE-CE-MS). A comparison between these platforms will be made and the great potential of EE-based strategies for enrichment of low-abundant metabolites and biomass-limited samples will be demonstrated.

23E 5:10 p.m. – 5:25 p.m.	TBD

PRESENTING AUTHOR: ***TBD***

CO-AUTHORS: ***TBD***

TBD

NEW FRONTIERS

SESSION 24: NOVEL APPLICATIONS	Wednesday, June 26	3:45 p.m. – 5:30 p.m.

24A Session Keynote 3:45 p.m. – 4:15 p.m.	Metabolomics: Central Approach for Study of the Exposome

PRESENTING AUTHOR: ***Dean Jones, Emory University, United States***

CO-AUTHORS: ***Edward T. Morgan, Choon-Myung Lee, Grant Singer, Ken Liu, Shuzhao Li, Young-Mi Go, Gary W. Miller***

Most human disease occurs through interactions of environmental exposures with an individual's genome. The exposome is defined globally to include dietary, pharmacologic, industrial and personal use products to which an individual is exposed. Metabolomics methods are very powerful because they provide means to capture biologic responses to exposures. In many cases, metabolomics also supports detection and quantification of xenobiotic chemicals and their metabolites. This latter finding has allowed development of an analytical framework in which metabolomics connects external exposures, internal body burden of chemicals and their metabolites, biologic responses to exposure, and health and disease outcomes. Examples of this central role of metabolomics in environmental health research will be provided in the presentation. The talk will also summarize new efforts, as part of the Chemical Identification Discovery Cores of the US National Institutes of Health Common Fund Metabolomics Consortium, to identify large numbers of un-identified low abundance mass spectral signals found in biospecimens. In this research, we are using xenobiotic detoxification enzymes to develop tools to rapidly generate and confirm identities of such xenobiotic metabolites. We start with knowledge that about 80 xenobiotic transformation systems occur in mammals. Prior knowledge of these systems allows creation of reconstituted cell-free systems for selective biotransformation reactions and also creation of stably transfected cell lines with over-expression of specific biotransformation systems. By creating arrays of these biotransformation systems in a 96-well format, we have found that we can simultaneously generate a series of xenobiotic metabolites. Because xenobiotic metabolites of each biotransformation reaction can be predicted, analysis of reaction products using liquid chromatography with high-resolution mass spectrometry and targeted MSn supports high-throughput xenobiotic metabolite identification. Studies with well characterized xenobiotics with known metabolites establish the utility of the approach and also demonstrate generation of previously uncharacterized xenobiotic metabolites. With creation of 384-well and 1536-well formats, we anticipate that the method can support mega-scale metabolite identification for large (>10,000) chemical libraries.

24B 4:15 p.m. – 4:35 p.m.	Metabolomes of mitochondrial diseases and inclusion body myositis patients: treatment targets and biomarkers

PRESENTING AUTHOR: ***Vidya Velagapudi, FIMM, University of Helsinki, Finland***

CO-AUTHORS: ***Buzkova Jana, Nikkanen Joni, Ahola Sofia, Hakonen Anna H, Sevastianova Ksenia, Hovinen Topi, Yki-Järvinen Hannele, Pietiläinen Kirsi H, Lönnqvist Tuula, Velagapudi Vidya, Carroll Christopher J, Suomalainen Anu***

Mitochondrial disorders (MDs) are inherited multi-organ diseases with variable phenotypes. Inclusion body myositis (IBM), a sporadic inflammatory muscle disease, also shows mitochondrial dysfunction. We investigated whether primary and secondary MDs modify metabolism to reveal pathogenic pathways and biomarkers. We investigated metabolomes of 25 mitochondrial myopathy or ataxias patients, 16 unaffected carriers, six IBM and 15 non-mitochondrial neuromuscular disease (NMD) patients and 30 matched controls. MD and IBM metabolomes clustered separately from controls and NMDs. MDs and IBM showed transsulfuration pathway changes; creatine and niacinamide depletion marked NMDs, IBM and infantile-onset spinocerebellar ataxia (IOSCA). Low blood and muscle arginine was specific for patients with m.3243A>G mutation. A four-metabolite blood multi-biomarker (sorbitol, alanine, myoinositol, cystathionine) distinguished primary MDs from others (76% sensitivity, 95% specificity). Our omics approach identified pathways currently used to treat NMDs and mitochondrial stroke-like episodes and proposes nicotinamide riboside in MDs and IBM, and creatine in IOSCA and IBM as novel treatment targets. The disease-specific metabolic fingerprints are valuable "multi-biomarkers" for diagnosis and promising tools for follow-up of disease progression and treatment effect.

24C 4:35 p.m. – 4:50 p.m.	Boosting anti-doping screening through metabolomics

PRESENTING AUTHOR: ***Luca Narduzzi, Oniris – Laberca, France***

CO-AUTHORS: ***Gaud Dervilly, Alexandre Marchand, Michel Audran, Bruno Le Bizec, Corinne Buisson***

The World Anti-Doping Agency (WADA) prohibits the use of recombinant analogs of human hormones in any sport competition. Nonetheless, their use is suspected to be widespread among professional athletes who intake hormones in micro-doses, to obtain mild but continuous anabolic effects while reducing the detectability window. In particular, recombinant Growth Hormone (recGH) detection is very challenging: its turnover is within 24 hours after injection, with athletes' age and hormonal profile as strong confounding factors. Any hormonal treatment on humans has, by principle, an impact on metabolism. In this proof-of-principle study, applying LC-HRMS based metabolomics and lipidomics workflows on human bio-fluids (urine/plasma) we intended to trace indirectly recGH administration to (i) highlight specific patterns associated to treatment and (ii) investigate potential biomarkers. The study included a cohort of 16 well-trained volunteers split in two equal groups administered with 1) micro-doses of erythropoietin (EPO) or 2) micro-doses of EPO + recGH. The two groups showed different metabolic profiles, especially in their plasma lipids. The analysis also evidenced high intra-group variability, indicating a putative subject-specific effect of the hormones. To solve this issue, we normalized the data versus the time 0, therefore we could evidence the subject-specific profile associated to the treatment while tracking longitudinally "what" and "when" has been taken. Biomarkers identification highlighted several metabolic pathways affected by hormones intake, including triglycerides catabolism and amino-acids turnover. On the contrary, WADA-approved recGH tests were not able to individuate recGH intake. Metabolomics seems to be a promising tool to screen for illicit recGH administration.

SESSION 24: NOVEL APPLICATIONS	Wednesday, June 26	3:45 p.m. – 5:30 p.m.

24D 4:50 p.m. – 5:10 p.m.	A Novel Metabolomics Method for Quantitative Analysis of over 1,000 Metabolites

PRESENTING AUTHOR: *Guowang Xu, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, China*

CO-AUTHORS: *Wangjie Lv, Lichao Wang, Qiuhui Xuan, Qingqing Wang, Xinjie Zhao, Chunxiu Hu, Xianzhe Shi, Xinyu Liu*

Metabolomics is the science of studying endogenous small molecular metabolites. It has been developed since the end of last century and has made great progress in the fields of diseases, pharmaceuticals, agriculture, environment, etc. The most typical method is non-target method, it is hoped that "all" metabolites can be panoramically analyzed without bias. At this moment, thousands to tens of thousands of features can be obtained by ultra high-performance liquid chromatography (UHPLC)-mass spectrometry (MS), and hundreds of metabolites can be identified. Because of the complexity of metabolome, it is far from meeting the needs of systems biology research. It would be much better if we could increase the number of metabolites detected quantitatively to nearly 2,000. From an economic point of view, of course, the fewer methods the better. In view of this, this study focuses on how to develop novel LC-MS-based analytical methods for the quantitative detection of more than 1,000 metabolites. Following three scientific problems are covered, 1. Analytical peak capacity extending by using two-dimensional LC; 2. Quantitative ion-pair selection by using the computational metabolomics algorithm and SWAT method; 3. Metabolic profiling annotation and peak identification by studying structure-retention and structure- fragment relationships under the assistance of standard samples and Internet databases. Through the above measures, we have established a novel metabolomics method to quantitatively analyze more than 1,000 metabolites in biological samples (body fluid, tissue and cells). This report will show the latest results of this study.

24E 5:10 p.m. – 5:25 p.m.	Post-mortem changes in the metabolomic profiles of animal and human tissues

PRESENTING AUTHOR: *Ekaterina Zelentsova, International Tomography Center SB RAS, Novosibirsk, Russia; Novosibirsk State University, Russian Federation*

CO-AUTHORS: *Lyudmila Yanshole, Vadim Yanshole, Olga Snytnikova, Yuri Tsentalovich*

The analysis of post-mortem metabolomic changes in biological fluids opens the way to develop new methods for the estimation of post-mortem interval (PMI) which appears to be an actual problem in forensic medicine. Also, for humans, autopsy materials are often used as control tissues due to the inaccessibility of the tissues of a healthy patient (brain, eye tissues and others). For the correct use, a detailed description of the changes in the metabolomic content of autopsy tissues should be investigated. The combination of modern research methods - high frequency 1H NMR and high-resolution LC–MS – was used in this work. The quantitative levels of 61 metabolites in the rabbit and human serum, aqueous and vitreous humors (AH, VH) at different PMIs have been measured. The obtained results indicate the advantage of ocular fluids, AH and VH, over blood serum for the search of biochemical markers for the PMI estimation: the post-mortem metabolomic changes in the ocular fluids proceed slower than in blood, and the data scattering is lower. These changes are governed by two major factors: the degradation of the ocular tissues and the diffusion of metabolites from the body vascular systems into ocular fluids. Among the metabolites whose concentrations increase with time, the most significant and linear growth is found for glycerol (rabbit), betaine (human), and hypoxanthine, choline (both). The high potential of choline, glycerol and betaine as biomarkers for the PMI estimation is revealed in the present work for the first time. Supported by RFBR (Projects N°18-34-00137, 19-34-80008)

SESSION 25: RESPIRATORY DISEASES	Thursday, June 27	8:45 a.m. – 10:00 a.m.

25A Session Keynote 8:45 a.m. – 9:15 a.m.	Omega-6 polyunsaturated fatty acid metabolites are associated with vitamin D levels in early life

PRESENTING AUTHOR: ***Jessica Lasky-Su, Brigham and Women's Hospital, United States***

CO-AUTHORS: ***Mengna Huang, Rachel S. Kelly, Su H. Chu, Priyadarshini Kachroo, Kathleen Lee-Sarwar, Augusto A. Litonjua, Scott T. Weiss***

Vitamin D is an important regulator of the immune system and low vitamin D levels are associated with a multitude of immunological disorders encompassing a broad range of diseases. The agnostic approach of untargeted metabolomics offers the opportunity to comprehensively examine changes in the metabolic profile associated with variations in vitamin D levels. The current study included the children who participated in Vitamin D Antenatal Asthma Reduction Trial, for whom plasma 25-hydroxyvitamin D [25(OH)D] and plasma metabolomics profiles were available at age 1 (n=451) and age 3 (n=407) years. Metabolomic profiling was conducted using ultrahigh-performance liquid chromatography and tandem mass spectrometry. After quality control, 624 known metabolites remained. We assessed the associations between individual metabolites and 25(OH)D level using linear regression adjusting for potential confounders. After correction for multiple comparisons, seven metabolites were significantly associated with 25(OH) D levels at both age 1 and age 3, including decreased concentrations of three members of the n-6 polyunsaturated fatty acid (n-6PUFA) metabolism pathway [linoleate(18:2), arachidonate(20:4), and docosapentaenoate(22:5)]. These n-6PUFA-metabolite-25(OH)D associations were replicated in older children from the Childhood Asthma Management Program($p<0.05$). This analysis is the first to characterize the metabolic profile associated with 25(OH) D levels at two time points in early life, with replication in an independent population. The association between increased 25(OH)D levels and decreased concentrations of multiple n-6PUFA metabolites is important as both are involved in inflammatory processes, and evidence from cell and animal studies has demonstrated plausible biological mechanisms where the active form of vitamin D may influence n-6PUFA metabolism.

25B 9:15 a.m. – 9:40 a.m.	Comprehensive metabolomic and immulogical profiling of Asthma COPD Overlap (ACO)

PRESENTING AUTHOR: ***Nilanjana Ghosh, School of Medical Science and Technology, India***

CO-AUTHORS: ***Priyanka Choudhury, Sushmita Roy Chowdhury, Rintu Banerjee, Parthasarathi Bhattacharyya, Koel Chaudhury***

Asthma chronic obstructive pulmonary disease (COPD) overlap, termed as ACO, is a complex heterogeneous disease without any clear diagnostic or therapeutic guidelines. The pathophysiology of the disease, its characteristic features and existence as a unique disease entity remain unclear. The present study aims to determine whether ACO has a distinct metabolic and immunological mediator profile in comparison to asthma and COPD. The study was conducted on two different patient groups, the discovery and validation cohort. The discovery phase patient cohort consisted of (i) controls= 33 (ii) asthma=34 (iii) COPD=30 and (iv) ACO=35. For the validation phase (i) asthma=32 (iii) COPD=32 and (iv) ACO=40 were considered. Serum and exhaled breath condensates (EBC) were collected from all the subjects and characterized using nuclear magnetic resonance (NMR) spectrometry and gas chromatography mass spectrometry (GC MS) based metabolomics. The immunological mediator profiling (Th1, Th2, Th17 cytokines and soluble immune mediators) was assessed using quantikine ELISA kits and magnetic bead based multiplexing assays. Multivariate and univariate analysis of NMR and MS metabolomics of combined serum and EBC profiles indicated dysregulated metabolites in ACO patients when compared with both asthma and COPD, followed by validation in a fresh cohort of patients. Our findings suggest that ACO has an enhanced energy and metabolic burden associated when compared to asthma and COPD. ACO also exhibits altered inflammatory and immune profiles than any of the parent diseases. It is anticipated that our results will stimulate researchers to further explore ACO and unravel the pathophysiological complexities associated with the disease.

25C 9:40 a.m. – 9:55 a.m.	The relationship between the maternal metabolome and childhood asthma: results from the Vitamin D Antenatal Asthma Reduction Trial (VDAART)

PRESENTING AUTHOR: ***Mengna Huang, Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School, United States***

CO-AUTHORS: ***Rachel S. Kelly, Su H. Chu, Priyadarshini Kachroo, Augusto A. Litonjua, Scott T. Weiss, Jessica Lasky-Su***

Several prenatal and perinatal factors have been implicated in the development of childhood asthma, including maternal smoking, diet, and pregnancy complications. The maternal metabolome during pregnancy may therefore provide valuable mechanistic insights and novel hypotheses in asthma pathophysiology. This analysis included non-smoking pregnant women and their children who participated in VDAART, a randomized placebo-controlled trial to determine the effect of prenatal vitamin D supplementation on offspring asthma (ClinicalTrials.gov identifier: NCT00920621). Asthma outcomes were defined as physician-diagnosed asthma or recurrent wheezing by age 3 and 6 years. Metabolomic profiling in 775 mothers at 10-18 weeks gestation was conducted using ultrahigh-performance liquid chromatography-tandem mass spectrometry. After quality control 734 known metabolites remained. We assessed the prospective associations between individual maternal plasma metabolites and childhood asthma/wheezing using multivariable logistic regression models. In 719 mother-child pairs, top maternal metabolites associated with asthma/wheezing by age 3 were 2-aminoadipate (P-value=8.91e-4), tyramine O-sulfate (P-value=1.05e-3), and vanillactate (P-value=3.02e-3). Vanillactate and 2-aminoadipate were also associated with asthma/wheezing by age 6 (n=775, P-values=2.35e-3, 4.07e-3). Among 25 and 18 metabolites associated with asthma/wheezing by age 3 and 6 respectively (P-value<0.05), thyroxine, 3-methylcatechol sulfate, and theophylline were also common. 2-aminoadipate and thyroxine had positive, while others demonstrated inverse relations with the outcomes. We identified several prenatal metabolites associated with childhood asthma/wheezing. Previously linked to diabetes and atherosclerosis, 2-aminoadipate is a lysine oxidation product by myeloperoxidase in inflammation. Theophylline, a caffeine metabolite and known asthma therapy, has bronchodilatory and anti-inflammatory effects. Validation in similarly-designed independent cohorts is needed to confirm these associations.

SESSION 26: MODEL ORGANISMS | Thursday, June 27 | 8:45 a.m. – 10:00 a.m.

26A Session Keynote 8:45 a.m. – 9:15 a.m. — Can Stage-Specific Metabolites be Recovered from Mixed-Stage C. elegans Cultures?

PRESENTING AUTHOR: *Arthur Edison, University of Georgia, United States*

CO-AUTHORS: *Sicong Zhang, Amanda O. Shaver, Brianna M. Garcia, Gonçalo J. Gouveia, Pam S. Kirby, Lauren M. McIntyre, Erik C. Andersen, Arthur S. Edison*

The model organism Caenorhabditis elegans develops in ideal conditions through six distinct developmental stages over its approximately three-day life cycle. Under unfavorable conditions, it can enter an alternative developmental stage called dauer, which can persist for months and is specialized for dispersal to a more optimal environment. Each developmental stage is unique in terms of gene expression, cell number, cuticle structure, and metabolism. Methods are available to synchronize cultures in order to isolate stage-specific nematodes, but these methods are time-consuming and add extra steps with harsh conditions that can potentially disrupt normal development. Furthermore, synchronization complicates large-scale discovery metabolomics, as the choices are to either focus on one specific stage or perform a synchronization step for each given life-cycle stage thereby increasing handling time. We are using both natural diversity and targeted genetic mutations in C. elegans for a large-scale NIH Common Fund project to identify unknown metabolites enabled by genetics and quantum chemistry. In an attempt to enable experiments on mixed stage populations, we are developing methods that will deconvolve mixtures in both LC-MS and NMR data by leveraging a series of stage specific and mixed stage experiments. Our approach would allow us to identify C. elegans developmental stages within a mixed-stage population using a large-particle flow cytometer. Each stage can then be associated with LC-MS or NMR features or vice versa. We will present progress on this approach using the N2 reference strain and discuss how it can be extended to other genotypes.

26B 9:15 a.m. – 9:40 a.m. — Bringing together what belongs together – Bridging the Caenorhabditis elegans model organism database, genome scale model and metabolite databases

PRESENTING AUTHOR: *Michael Witting, Research Unit Analytical BioGeoChemistry, Helmholtz Zentrum München, Germany*

CO-AUTHORS: *Jake P. N. Hattwell, Horst Joachim Schirra, Karen Yook, Keeva Cochrane, Claire O'Donovan*

Despite considerable efforts, information on Caenorhabditis elegans metabolism, its genes, proteins and metabolites is currently scattered across different databases. The WormJam (short for Worm Jamboree) community is a world-wide community of researchers who aim to rectify this situation by constructing and curating a high-quality consensus genome-scale metabolic network (GSMN) of this important biomedical model organism. The resulting WormJam GSMN is currently one of the best curated metabolic models for C. elegans. WormJam collaborates with WormBase (www.wormbase.org), the premier repository for C. elegans genes, proteins, phenotypes, and related information. We have recently started to also add metabolites to this repository. The WormJam model uses ChEBI as primary information and deposition source for metabolite structures, and together with the WormJam community we have started towards full coverage of the C. elegans GSMN with metabolite structures. In addition, we are working on the integration of RheaDB to allow comparison of metabolic reactions between other organisms and C. elegans. Furthermore, we are collaborating with MetaboLights to link metabolomics raw and reference data. Here we present an application example using a case study on C. elegans sphinoglipids and their analysis using UPLC-UHR-ToF-MS. We started from the correction and curation of sphingolipid-related reactions in WormJam as well as curation of structures, collection of reference MS and MS/MS. We are aiming to further develop the WormJam model and connected database as a comprehensive knowledge base for future investigations into the metabolism of C. elegans.

26C 9:40 a.m. – 9:55 a.m. — The use of mitochondrial metabolomics in discovering the molecular function of a mitochondrial membrane protein

PRESENTING AUTHOR: *Daqiang Pan, Centre for Biological Systems Analysis, University of Freiburg, Germany*

CO-AUTHORS: *Caroline Lindau, Simon Lagies, Michael Rodamer, Nils Wiedemann, Bernd Kammerer*

Yeast Saccharomyces cerevisiae SYM1 is an ortholog of the human MPV17 gene whose mutation causes mitochondrial DNA depletion syndrome. Sym1 protein is located in the inner mitochondrial membrane and its deletion results in impaired mitochondrial bioenergetic functions and morphological features under stress conditions. However, the functions of both Mpv17 and Sym1 have not been clearly characterized. Recently, compartment-specific metabolic alterations to mutations or inhibition of mitochondrial proteins were revealed by analyzing isolated mitochondria, opening new doors for uncovering the function of Sym1. Therefore, mitochondria and the corresponding cytoplasmic fraction of wild type and Δsym1 cells were isolated through differential centrifugation and subjected to metabolomic analysis. TCA cycle intermediates were accumulated overall in Δsym1 cells. This correlates with the results of Dallabona et al., which showed severe OXPHOS defects of Δsym1 under stress conditions. Meanwhile, accumulated saccharopine and reduced lysine suggested an interrupted lysine biosynthesis, which was proved by incubating yeast cells without the supply of lysine. Interestingly, pyrimidine biosynthesis intermediates, carbamoyl-aspartate and orotic acid, were reduced in Δsym1 cells. This situation became worse when the growth temperature was shifted, after incubation for eight hours, from 30 °C to 37 °C for eight hours, indicating that sym1 may play a role in pyrimidine biosynthesis. This is the first time that metabolomics was applied to determine the molecular function of the mitochondrial inner membrane protein sym1, which gives new aspects in understanding its function.

TECHNOLOGY

SESSION 27: GENOME-SCALE MODELING	Thursday, June 27	8:45 a.m. – 10:00 a.m.

27A Session Keynote 8:45 a.m. – 9:15 a.m.	Computational modelling of host-microbiome co-metabolism

PRESENTING AUTHOR: ***Ines Thiele, National University of Ireland, Ireland***

Computational modelling of human and microbial metabolism has gained increasing attention for phenotypic characterization. Such modelling is achieved by assembling in a bottom-up manner a high-fidelity computational representation of an organism's metabolic network based on genomic, biochemical, and physiological data. We have developed a semi-automated pipeline for the assembly of high-fidelity microbial metabolic networks and applied this pipeline to generate a collection of more than 800 gut microbial metabolic networks. We can now combine these metabolic networks, e.g., based on metagenomic data, to predict emergent metabolic capabilities of the microbial community and their potential effect on the human host. To that end, we built whole-body metabolic models of a male (deemed Harvey) and a female (deemed Harvetta), which describe accurately the metabolism occurring in 28 organs. Importantly, these whole-body models can be expanded to include the strain-resolved metabolic models of gut microbes. I will demonstrate how different microbial composition leads to differences in host metabolism, such as the capability to produce important neurotransmitters in the brain and flux through liver enzymes, with implications for the gut-brain axis as well as for microbiome-mediated liver toxicity. The predictions were consistent with our current understanding but also highlighted that different microbiota composition can lead to high inter-person variability. I envisage the microbiome-associated whole-body metabolic models will usher in a new era for research into causal host-microbiome relationships and greatly accelerate the development of targeted dietary and microbial intervention strategies.

27B 9:15 a.m. – 9:40 a.m.	Metabollic footprint of Parkinson's Disease: integration of patient-derived X-omics data with a human genome-scale model.

PRESENTING AUTHOR: ***Agnieszka Wegrzyn, LACDR, Leiden University, Netherlands***

CO-AUTHORS: ***Agnieszka Wegrzyn, Edinson Lucumi, Alida Kindt, Cornelius Willacey, German Preciat, Jennifer Modamio Chamarro, Zhi Zhang, Evan G. Williams, Rashi Halde, Javier Jarazo, Paul Wilmes, Enrico Glaab, Jens Schwamborn, Amy Harms, Thomas Hankemeier, Ronan M.T. Fleming***

The exact molecular mechanism of Parkinson's disease (PD) is currently unknown. However, it is believed that metabolic malfunctions play an essential role in the development of PD. Several genetic risk factors linked to bioenergetic alterations were identified, including mutations in a mitochondrially targeted, neuroprotective serine/threonine protein kinase PTEN-induced kinase (PINK1). However, the link between PINK1 mutations and the metabolic deregulation that leads to the development of PD remains mostly unknown. Here, we explore the metabolic footprint of a PINK1 mutation by creating a PINK1-specific metabolic model based on integrated patient-derived x-omics data. To provide patient-specific constraints of the PINK1-Q456X mutation for the model, we generated transcriptomic (RNAseq), untargeted proteomics, and quantitative exometabolomic (targeted mass spectrometry) data from in vitro cultures of patient iPSC-derived dopaminergic neurons carrying this mutation. Each PINK1-Q456X neuronal line was paired with its isogenic control, in which the PINK1 gene was corrected. Additionally, cell lines derived from healthy volunteers were used for comparison. With this x-omics data, we generated constraint-based, genome-scale, patient-specific in silico models of dopaminergic neuronal metabolism affected by the PINK1 mutation (iPINK1). Furthermore, we created patient-specific control models reflecting the metabolism of isogenic controls (iPINK1iso) and healthy volunteers (iPINK1ctrl). Our models are being used to predict targets for reducing mitochondrial dysfunction. We will check which non-trivial perturbations to reaction rate(s) can compensate for the effect of mutations in monogenic PD patients, or act to improve dysfunctional mitochondrial phenotypes.

27C 9:40 a.m. – 9:55 a.m.	Genome-wide metabolic modeling of human CD4+ T-helper cells differentiation unraveled the relative importance of ceramides

PRESENTING AUTHOR: ***Partho Sen, Postdoctoral Researcher, Finland***

CO-AUTHORS: ***Partho Sen, Alex M Dickens, Ubaid Ullah, Syed Bilal Ahmad Andrabi, Mohd Moin Khan, Tuomas Lindeman, Esko Kemppainen, Tanja Buchacher, Omid Rasool, Tuulia Hyötyläinen, Riitta Lahesmaa, Matej Oresic***

T-helper (Th) cells play a pivotal role in cell-mediated immunity. During the development, T cells undergo metabolic remodeling that is essential for orchestrating the action of other immune cells. In order to understand global metabolism during T-cell development, we developed genome-scale metabolic models (GEMs) for human Th1, Th2 and Th17 subsets and T-regulatory cells (iTreg)1. Meta-analysis of T-cell specific human transcriptomics datasets have identified 72 novel metabolic genes, corresponding to 355 reactions spanning various metabolic pathways. Reporter metabolites and pathway overrepresentation analysis suggested that T-cell activation induces gluconeogenesis, glutaminolysis, and lipid biosynthesis. Moreover, ganglioside (GA1, GMb) and N-acetylneuraminate (NANA) associated with sialyl-T antigen were significantly up-regulated in Th17 cells at 72 hours of initiation, while the glucosyl-, lactosyl- and galactosyl ceramides were down-regulated. On contrary, such trends were either reversed or absent in iTregs at this time-point. Our findings suggest that, ceramides are involved in the metabolic regulations and functioning of T-cells. It was also found that, Th subsets exhibit unique metabolic phenotype, already during early stages (72 h) of specification, thus playing a central role in guiding the fate of the cells. These results are being validated by Liquid Chromatography and Mass spectrometry (LC-MS)-based targeted metabolomics experiments. The findings from this study, provide a basis for modulation of human Th subsets crucial for immune responses under metabolically aberrant conditions and in immune-mediated disorders.

SESSION 28: MICROBIOME	Thursday, June 27	10:45 a.m. – 12:00 p.m.

28A Session Keynote
10:45 a.m. – 11:15 a.m.

Global Chemical Impacts of the Microbiome Include Novel Conjugated Bile Acids that Stimulate FXR

PRESENTING AUTHOR: ***Robert Quinn, Michigan State University, United States***

CO-AUTHORS: ***Alexei Melnik, Alison Vrbanac, Zsolt Bodai, Hera Vlakamis, Ting Fu, Julian Avila-Pacheco, Morgan Panitchpakdi, Mingxun Wang, Ron Evans, Manuela Raffatellu, Curtis Huttenhower, Sarkis Mazmanian, Rob Knight, Pieter Dorrestein***

A mosaic of cross-phyla chemical interactions occurs between all metazoans and their microbiomes. These microbial residents in humans are increasingly well characterized, but we have yet to elucidate the breadth of the chemical diversity the microbiome contributes. We used untargeted LC-MS/MS based metabolomics and the mass spectrometry database GNPS to assess the global metabolite differences between germ-free (GF) and colonized mice. Of the 7,913 molecules detected across 29 murine organs, 14.7% were unique to SPF. Unique microbial compounds included bile acids conjugated with the amino acids phenylalanine, tyrosine and leucine, which represent a new tertiary group of bile acids produced by the microbiome that have eluded discovery in the 170 years of research on bile chemistry. Culturing human gut isolates revealed that Clostridium bolteae was responsible for their production. These molecules were found in the upper GI tract of mice and elevated in abundance when fed high fat. Searching GNPS showed that they are also present in humans and elevated in individuals with inflammatory bowel disease (IBD). These novel conjugates strongly agonize the human FXR receptor, which is a global regulator of bile acid metabolism. Unlike the host produced glycine and taurine conjugates, these novel molecules could not be deconjugated by the microbiota, making their action on the FXR constitutive. FXR agonism in vivo reduced the overall production of bile, making these molecules microbial manipulators of bile acid metabolism. The discovery of these molecules opens up a new era in bile acid research linking these novel conjugates with human disease.

28B 11:15 a.m. – 11:40 a.m.

Exploring the microbiota-host epigenetics axis in female and male germ-free and conventional mice

PRESENTING AUTHOR: ***Joan Miró Blanch, Metabolomics Platform, IISPV & Department of Electronic Engineering, Universitat Rovira i Virgili, Spain***

CO-AUTHORS: ***Jordi Capellades, Alexandra Junza, Magdalini Serefidou, Claire Maudet, Aurélie Balvay, Pau Gama-Perez, Ignasi Forné, Pablo M. Garcia-Roves, Axel Imhof, Oscar Yanes***

Histone modifications are major regulators of the epigenetic machinery, due to their ability to modulate gene expression, DNA repair and chromatin structure. These post-translational modifications (PTMs) are dynamically regulated by specific modifying enzymes whose activities require metabolites that either serve as substrates, cofactors or act as activators/inhibitors. The level of these metabolites depend on host metabolism and environmental factors, including the metabolic activity of gut microbiota. Here, liver tissue from male and female germ-free (n=20) and conventional (n=20) mice has been studied to gain new knowledge on the epigenetic regulation at the interplay between host and gut microbiota metabolites. To do this, we have implemented a targeted metabolomic analysis by LC-QqQ MS covering >30 epigenetically relevant metabolites playing a regulatory role in histone acetylation, methylation, phosphorylation, ADP-ribosylation, and N-acetylglucosamination reactions. At the same time, we have quantified histone PTMs by LC-MS/MS in data dependent acquisition mode. Remarkably, we observed that S-Adenosyl-Methionine (SAM) is altered in the absence of microbiota and this highly correlates with changes in lysine methylation status of histones H3 and H4. These results were further validated in diet-induced obese mice presenting changes in the gut microbiota composition. Furthermore, ongoing RNAseq experiments will hopefully reveal alterations in the liver transcriptional profile associated with the absence (germ-free) or dysbiosis of the microbiota induced by a high fat diet.

28C 11:40 a.m. – 11:55 a.m.

Individual Variations in Plasma Metabolites are Driven by Diet, Genetics and Gut Microbiome

PRESENTING AUTHOR: ***Lianmin Chen, University Medical Centre Groningen, Netherlands***

CO-AUTHORS: ***Daria V. Zhernakova, Mihai G. Netea, Folkert Kuipers, Cisca Wijmenga, Alexandra Zhernakova, Jingyuan Fu***

Plasma metabolome and its inter-individual variation underlie individual's susceptibility of various complex diseases. Diet, genetics, and the gut microbiome are determinants of human metabolic status. However, their relative contributions to variation in plasma metabolome remain elusive. Here, we present a comprehensive host-microbe-diet interaction analysis on over 1000 plasma metabolites in a population-based Lifelines-Deep cohort (n=1,440), for each individual we had completed information on the gut microbial taxonomic and functional composition, genetic background, blood metabolites, dietary factors, medication and clinical phenotypes. Our study found diet, genetics and gut microbiome could explain 9%, 8% and 14% of variation respectively. Moreover, medication explained 4% and host physiological status could explain 10%. These factors together contributed 39% of variation in plasma metabolome. On individual metabolite level, we reported 88 associations to genetics, 369 to the gut microbial species, 7884 to microbial pathways and 261 to dietary factors at FDR<0.05. Notable, 61 metabolites were associated to all of them, indicating host-microbe-diet interactions. These metabolites are enriched for lipids, amino acids, carbohydrates, bile acids and fatty acids, which in turns can affect host health and disease. Notable, some microbial metabolites synthesized or modulated by the gut microbiome are linked not only to human derived metabolites but also linked to host immune profile. Taken together, this study reveals important evidence for a significant role of the gut microbiome in host metabolism and its interaction with diet and host genetics, providing clues for future applications in personalized medicine.

PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

SESSION 29: PLANT APPLICATIONS 2	Thursday, June 27	10:45 a.m. – 12:00 p.m.

29A Session Keynote 10:45 a.m. – 11:15 a.m.	A novel plant-microbiome co-culturing system reveals key associations of specific metabolites with plant growth and stress tolerance

PRESENTING AUTHOR: *Sanjay Swarup, National University of Singapore, Singapore*

CO-AUTHORS: *Gourvendu Saxena, Ee Yong Lian, Pavagadhi Shruti, Miko Poh Chin Hong, Yeap Yoon Ting*

Plants coexist with microbes as holobionts, sharing mutually beneficial functions, which can be engineered to produce ecologically and environmentally favorable outcomes. The rhizosphere microbial community, which occupies the niche developed by the gradients of root exudates in the rhizosphere region is of particular interest due to its probable direct role in providing specific factors for plant growth. However, the lack of a model rhizosphere microbial community and its metabolite exchanges with the host has limited the much needed mechanistic understanding of plant-microbe interactions in the rhizosphere. To fill this gap, we have developed a novel plant holobiont gnotobiotic system with specific characteristics of (i) co-culturing of model plant, Arabidopsis thaliana or related Brassica leafy vegetables, with a highly complex microbial community of hundreds of taxa; (ii) easy real-time phenotyping of plant growth; (iii) metabolites recovery from root surface or root-microbiome interface and (iv) microbiome recovery from the co-culture system. Live exudation from plants supports a highly complex microbiome community of several hundred taxa and recovery of highly complex metabolites mixture. High Resolution and Accurate Mass –mass spectrometry using an Orbitrap system led to the identification of more than 15,000 de-isotoped molecular features. Using this system, we show a very specific plant growth effect by the enriched rhizosphere microbial community on the plants through different distinct metabolic pathways, when compared with the bulk-soil microbial community. The mechanistic understanding from this research framework can be used on specific crops to enhance the yields without adding carbon or water footprint to the system.

29B 11:15 a.m. – 11:40 a.m.	An untargeted LC-MS based work-flow for the structural characterization of biological polyesters

PRESENTING AUTHOR: *Rebecca Dauwe, Université de Picardie Jules Verne, France*

CO-AUTHORS: *Benjamin Thiombiano, Roland Molinié, Paulo Marcelo, Eric Gontier, François Mesnard*

We developed an LC-MS based strategy to characterize fragments of the lignin macromolecule, a complex phenolic polyester that accumulates in flax seed integuments. The polyester was subjected to a dynamic alkaline hydrolysis process that was stopped after a series of increasing reaction times, and the hydrolysates were analyzed by LC-MSn. Analysis of the final hydrolysates led to the identification of 31 monomeric subunits. For the characterization of the partial hydrolysates, we show that the annotation of pairs of related LC-MS features, based on m/z differences, strongly facilitates their characterization. Using self-organizing maps, we first preselected as candidate ester-containing compounds those features that transiently appeared during the dynamic hydrolysis process. Among all possible pairs of the preselected features, we revealed 46 overrepresented m/z differences that corresponded to the addition of identified macromolecule monomers or of theoretically possible oligo-esters built of two or three monomeric units. In order to reveal the features that represent different fragments of the same lignan macromolecule, a candidate substrate-product pair network connecting the selected features by the annotated overrepresented m/z differences was built. The network information was then combined with the interpretation of MSn data to elucidate the stuctures of the saponification fragments. In total, 120 distinct oligo-esters, consisting of up to 6 lignan macromolecule monomers could be characterized. These results allowed us to further elaborate the existing structural model of the lignan macromolecule. Our network approach shows promising to tackle the structural characterization of different types of complex plant polyesters, such as suberin and cutin.

29C 11:40 a.m. – 11:55 a.m.	NMR-MS metabolomics reveals sulfonation in the Salicaceae

PRESENTING AUTHOR: *Clarice Noleto-Dias, Rothamsted Research, United Kingdom*

CO-AUTHORS: *Claudia Harflett, Jane L. Ward, Michael H. Beale*

The Salicaceae family is characterised by the presence of phenolics, including the glycosides of the salicinoid sub-group and different types of flavonoids. Rothamsted Research is home to the National Willow Collection, comprising 1500 different accessions. In addition, large scale mapping populations have been developed. A metabolomics screen of a diversity panel comprising mapping population members and their parental lines suggested that a particular population (MpF) was dominated by metabolites not observed in other Salix species. Fractionation via HPLC was performed to isolate all the novel compounds present in the sample. Five sulfated compounds were isolated and their structures elucidated by LC-HRMS-MS and 1D and 2D-NMR. Four compounds were found in leaf and stem tissues of S. alberti x S. integra x S. suchowensis hybrid (NWC901), a parent of MpF, and these were characterised as taxifolin-7-sulfate, dihydrokaempferol-7-sulfate, eriodictyol-7-sulfate and narigenin-7-sulfate. Although sulfation of natural products is not rare, and occurs often in mammalian metabolism, most examples from the plant world concern sulfated flavonoids from flavonol and flavone subclasses, different to the flavanonols and flavanones reported herein. An additional compound, salicin-7-sulfate, was also identified in the LC-MS datasets of stem tissue samples harvested at the dormant stage of some of the 86 pure Salix genotypes. Among these lines, no correlation between the amount of salicin and its sulfated form could be detected. We will present data on compounds that have not been previously described in plants and discuss the implications of their presence in willow, a plant traditionally used in herbal medicine.

SESSION 30: METABOLITE IDENTIFICATION 2	Thursday, June 27	10:45 a.m. – 12:00 p.m.

30A Session Keynote 10:45 a.m. – 11:15 a.m.	Correlation-based deconvolution (CorrDec) method for data-independent acquisition mass spectrometry

PRESENTING AUTHOR: *Ipputa Tada, Department of Genetics, SOKENDAI (Graduate University for Advanced Studies), Japan*

CO-AUTHORS: *Romanas Chaleckis, Hiroshi Tsugawa, Isabel Meister, Pei Zhang, Craig E. Wheelock, Masanori Arita*

Metabolite identification is still a major challenge in untargeted metabolomics. Data-independent acquisition mass spectrometry (DIA-MS) is essential for information-rich and reproducible analysis of metabolome, but the acquired full-scan MS2 spectra are highly complex and pose harder challenges for annotation and identification. We have developed a new deconvolution method (CorrDec) that exploits the correlation between peak intensities of precursor and fragment ions in multi-sample studies measured with All Ion Fragmentation (AIF). Its main advantage is the discrimination of completely co-eluting, low-abundance compounds; this is the first software approach that systematically disentangles clean MS2 spectra from full-scan AIF data. The CorrDec was implemented in our MS-DIAL software (version 3.32 or later) and its performance was rigorously assessed with the original, retention-time based MS-DIAL deconvolution (MS2Dec) using a dilution series of chemical standards and biological samples. Both methods function in complement, and the benefit of CorrDec is extraction of cleaner, i.e., more library-matching MS2 spectra than MS2Dec when multiple samples are available. In this presentation, we report detailed performance comparison of both deconvolution methods and our randomized assessment using biological samples to estimate the required number of measurements for CorrDec. We also introduce interpretation of deconvoluted MS2 spectra of marginally identifiable metabolites, using co-eluting betaines as a model case.

30B 11:15 a.m. – 11:40 a.m.	Evaluation of molecular ionization propensities in different ionization modes: providing evidence for the presence of small molecules in synthetic blinded samples

PRESENTING AUTHOR: *Jamie Nunez, Pacific Northwest National Laboratory, United States*

CO-AUTHORS: *Sean Colby, Thomas Metz, Justin Teeguarden, Ryan Renslow*

For non-targeted metabolomics analysis, the relative ionization propensities of molecules analyzed using different ion sources (e.g. electrospray, ESI; atmospheric pressure photoionization, APPI) is not well understood. Improved understanding of ionization propensities would offer many advantages in metabolomics, such as (i) a priori selection of ionization modes based on suspected sample composition, (ii) the ability to reduce the number of candidate structures during data analysis by removing those less likely to ionize, and (iii) a reduced false discovery rate and increased confidence in identifications. Here, we will discuss a blinded analysis of synthetic chemical mixtures, each containing up to hundreds of unique compounds, as part of the U.S. EPA's Non-Targeted Analysis Collaborative Trial (ENTACT). We report how different ionization modes contributed to overall identifications, as well as how certain chemical properties (e.g., pKa, LogP, molecular mass, ring-count) and chemical substructures (e.g., heterocyclic rings and carboxyl, carboxamide, and phosphino groups) influence ionization propensities. For example, several chemical properties were found to be statistically significantly different for compounds identified using ESI or APPI, and in positive or negative ionization modes. Furthermore, we clustered compounds based on their chemical properties, chemical class, and chemical substructures, and found that certain properties and substructures were highly correlated with detection in specific ionization modes, while chemical class, derived from an automated ontology (ClassyFire) did not provide strong discriminating information. While similar analyses have been performed on a small scale in the past, our fully automated approach facilitated a large-scale analysis and provides a foundation for future studies.

30C 11:40 a.m. – 11:55 a.m.	Improved oxylipins and fatty acids identification by using LC-DTIM-MS

PRESENTING AUTHOR: *Sonia Liggi, Department of Biochemistry and Cambridge Systems Biology Centre, University of Cambridge, United Kingdom*

CO-AUTHORS: *Christine Hinz, John Fjeldsted, Julian L. Griffin*

The use of liquid chromatography coupled with drift tube ion mobility and mass spectrometry (LC-DTIM-MS) can help the characterization and identification of lipid species, reflecting the additional dimension provided by ion mobility which can improve separation of the numerous structural isomers in these molecular classes. Nonetheless, the analysis of oxylipins is particularly challenging given their physiological low abundance in biological samples and the narrow mass range occupied by this lipid category. To overcome these limitations, we developed a combined analytical-computational method for the analysis and identification of oxylipins and their precursor fatty acids. Herein, we focus on presenting the computational part of the workflow. More specifically, accurate mass, collision-cross section values in nitrogen (DTCCSN2) and retention times of 47 oxylipins and fatty acids standards were obtained after triplicate acquisition on a reversed-phase LC-DTIM-MS workflow, and integrated in an internal library subsequently used for the identification of these compounds in biological samples. Aside from directly identifying oxylipins and fatty acids, we were also able to flag species as similar to those in the internal library. These flags not only reduce the number of annotations from external databases, providing a lead for further characterization analyses, but also guide discovery studies of potentially novel species. The method was applied to several datasets of biological samples, such as Salmonella enterica serovar Typhimurium infected murine bone-marrow derived macrophages and thrombin activated human platelets, providing results in agreement with the literature and improving the identification of features otherwise annotated with a vast number of compounds.

Poster Abstracts

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*AWARD WINNERS

BIOMEDICAL

P-1 The impact of the Healthy New Nordic diet on the human urine metabolome as measured by 1H-NMR spectroscopy

PRESENTING AUTHOR: ***Alessia Trimigno, University of Copenhagen, Denmark***

CO-AUTHORS: ***Bekzod Khakimov, Søren Balling Engelsen***

More than a decade ago, chefs and scientists designed the New Nordic diet (NND). This diet was based on sustainable, local and nutritious foods. It mainly consisted in vegetables, fruits, berries, nuts, fish, shellfish and game meat. NND was proved beneficial on overweight people, i.e. decreasing body weight, reducing glycaemia, lowering blood pressure, and improving blood lipids. In this study, we investigated how the healthy NND affects the human urine metabolome and compared it with the effect of the Average Danish Diet (ADD). Urine samples from 142 centrally obese Danes were collected at baseline, after 6 months and 12 months of intervention. Subjects were randomly assigned to ADD or NND. Urine samples were analyzed through untargeted 1H-NMR spectroscopy and the acquired spectra were converted into a metabolite table using the novel Signature Mapping (SigMa) approach. The urine metabolite table was scrutinized using multivariate data analysis methods to reveal the impact of the different study design factors including diet, season, gender, and bodyweight change. NND samples revealed higher concentrations of glycine, betaine, glucose, TMAO and creatinine, whilst ADD urine had greater levels of tartrate, dimethyl sulfone and propylene glycol. The latter was also found to be associated in the NND group with the homeostatic model assessment for insulin resistance (HOMA). Results from this study corroborate previous information on the NND, indicating different effects on the metabolism of proteins and carbohydrates, and help in better understanding its actual impact on human health (e.g. glycaemia, inflammation) and on the gut microbiota metabolism.

P-2 Environmental cadmium exposure disrupted mitochondrial metabolome in human and mouse lungs

PRESENTING AUTHOR: ***Young-Mi Go, Emory University, United States***

CO-AUTHORS: ***Matthew R. Smith, Xin Hu, Jolyn Fernandes, Michael Orr, Doug Walker, David Neujahr, Dean P. Jones***

Cadmium (Cd) causes adverse health effects from occupational exposures and cigarette smoking. Lower level of environmental Cd exposure, principally from food, is a global health concern because of the long biological half-life of Cd (10-30 years) in humans and animals. Despite Cd toxicity at high dose exposures being well documented, the impacts of Cd exposures at low levels through dietary intake has only recently been characterized. Our previous study showed that lung Cd burden found in non-smoker's lung caused changes in the mouse lung metabolome with effects on airway reactivity, glycolysis and lipid metabolism, inflammation and profibrotic signaling. Our data further showed potentiated adverse reaction to H1N1 influenza virus and respiratory syncytial virus and worsened microbiome disruption by high-fructose diet. In the current study, we examined the effects of Cd on lung mitochondrial metabolome using ultrahigh-resolution mass spectrometry-based high-resolution metabolomics (HRM). Mitochondria (250 mg) isolated from lungs were analyzed for metabolome and metabolic pathway by HRM, and concentrations of Cd and other metals by Inductively Coupled Plasma Mass Spectrometry. The results showed that Cd caused alterations in large numbers of mitochondrial metabolites including fatty acids, carnitines and CoA, and disrupted fatty acid oxidation and energy metabolism. The results suggest that Cd accumulation by dietary exposure could be a potential pulmonary health risk involving disruption of mitochondria as a central mechanism in lung, specifically in fatty acid and energy metabolism and enhanced inflammation by mitochondrial H2O2 signaling.

P-3 Integration of proteomics and metabolomics data to stratify severe septic shock patients

PRESENTING AUTHOR: ***Manuela Ferrario, Politecnico di Milano, Italy***

CO-AUTHORS: ***Alice Cambiaghi, Eliandre de Olivera, Laura Brunelli, Roberta Pastorelli***

In this work, we examined plasma metabolome and proteome in patients with severe septic shock enrolled in the multicenter ALBIOS study (Albumin Italian Outcome Sepsis study, NCT007071225). The objective was to identify changes in the levels of metabolites involved in septic shock progression and to integrate this information with the variation occurring in proteins. Mass spectrometry based targeted metabolomics and untargeted proteomics allowed us to quantify absolute metabolites concentration and relative proteins abundance. We computed the fold change D7/D1 to take into account their variation from day 1 (D1) to day 7 (D7) after shock diagnosis. Patients were divided into two groups according to 28-day mortality. Two different elastic net logistic regression models were built: one on metabolites only and one on metabolites and proteins. Linear Discriminant Analysis and Partial Least Squares Discriminant Analysis were also implemented. A filter strategy was adopted to reduce the number of features. All the obtained models correctly classified the observations in the testing set. Our findings confirmed that non-survivors have different trend in plasma levels of lipid species in comparison to survivors, in line with previous works. Our approach showed that the proteins decisive in stratifying the patients are those related to the inflammatory response and the coagulation cascade, which are known to play an important role in septic shock progression, thus confirming the feasibility and robustness of our integrative approach. Integrating metabolomics data with proteomics one enabled to highlight key features associated with the outcome.

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*AWARD WINNERS

BIOMEDICAL

P-4 New insight in Metabolomics based study of Age Related Macular Degeneration (AMD): Lipoprotein profile and patients follow-up

PRESENTING AUTHOR: ***Matthieu Schoumacher, Uliege (CIRM), Belgium***

CO-AUTHORS: ***V. Lambert, S. Hansen, J. Leenders, B. Govaerts, B. Pirotte, J.-M. Rakic, A. Noël and P. de Tullio***

Age-related macular degeneration (AMD) is common disease and leading causes of vision loss among people aged 50 and older. Late-stage AMD (called exudative) is characterized by choroidal neovascularization (CNV) and results in complete loss of central vision acuity leading to severe visual impairment and legal blindness. Currently, AMD diagnosis relies on ophthalmologic exams and treatments of the exudative form using anti-angiogenic drugs targeting vascular endothelial growth factors (VEGF). Despite these advances, the identification of therapeutic treatments and related biomarkers are essentials. In this study, we applied a NMR-based metabolomics approach on a cohort of AMD patients and on a laser-induced murine CNV experimental model that mimics the pathology angiogenesis's development phase. Sera from controls and AMD patients (in active and non-active pathology's phases) and from induced and non-induced mice have been collected and submitted to a metabolomics study, using a multivariate approach. This approach allows differentiation between active and non-active AMD patients and between laser-induced and the control mice groups. Moreover, the discriminating spectral zones are the same in both human and mice's models, leading to the emergence of putative biomarkers of the exudative AMD. Among those, lipoprotein profile is interesting while some studies highlighted HDL cholesterol and oxidized LDL with early and para-inflammatory AMD stages. Our primary studies show similar evolution in lipoprotein profile through the different human and mice' groups. These data suggest that investigate lipoprotein profile could be the turning point in the pathologic process's comprehension occurring during the apparition and/or the development of pathologic CNV process.

P-5 Confirming the key role of glutathione in the mechanism of drug resistance to artemisinin-based antimalarials

PRESENTING AUTHOR: ***Dovile Anderson, Monash University, Australia***

CO-AUTHORS: ***Ghizal Siddiqui, Amanda De Paoli, Darren J. Creek***

Malaria treatment is reliant on artemisinin and its analogues in combination with partner drugs. Emerging resistance of Plasmodium falciparum parasites to artemisinin-based combination therapies (ACTs) reduces clinical effectiveness and threatens global malaria control efforts. Therefore investigations of the mechanisms of resistance are urgently required. It has been shown that slow parasite clearance following artemisinin treatment is associated with mutations in the PfKelch13 gene. Metabolomics analysis of these PfKelch13-mutant parasites has revealed increased levels of glutathione (GSH) and gamma-glutamyl cysteine compared to the artemisinin-sensitive line. GSH is known to play a pivotal role in anti-oxidative defence and other cellular processes within the parasite. In this study, the role of GSH in PfKelch13-mediated artemisinin resistance was investigated by inhibiting GSH biosynthesis pathways. Inhibition of GSH biosynthesis was achieved by pre-incubating resistant parasites with buthionine sulphoximine (a gamma-glutamylcysteine synthetase inhibitor) and sulfasalazine (a cysteine transport inhibitor), which restored sensitivity towards artemisinin to levels comparable to sensitive lines (40% survival decreased to 18%, $p < 0.05$). A targeted LCMS assay was developed and validated to measure the levels of free and oxidised thiols in parasites (sensitive and resistant) following GSH depletion. These findings give further insight into parasite detoxification mechanisms and confirm the key role of GSH metabolism in PfKelch13-mediated artemisinin resistance.

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*AWARD WINNERS

BIOMEDICAL

P-6 Metabolomics analysis of human serum for population-based characterisation of ageing in men

PRESENTING AUTHOR: ***Dakshat Trivedi, Mancehster Institute of Biotechnology, University of Manchester, United Kingdom***

CO-AUTHORS: ***Fredrick Wu, Roy Goodacre***

With increasing life expectancy in most developed countries, it is becoming essential that the promotion of healthy ageing is part of public health objectives. However, relatively little is known about the hormonal transitions in men compared to female at the onset and progression of ageing (Lee et al., 2009). In particular, the changes in the human metabolome during this phase of life are relatively under-studied. Metabolomics is aimed at measuring the totality of the metabolites within a particular biological system (Goodacre et al., 2004) be it blood, urine or cells. To determine the differentiating classes of metabolites of ageing men, serum from 384 ageing males of different ethnic origin i.e. South Asian (SA) (n=118), African Caribbean (AC) (n=118) and White European (WE) (n=118) were chosen for spectroscopic analysis. Subjects' BMI values (+/- 2) and age (+/- 1 year) were matched prior to analysis. 300uL serum was analysed with Raman spectroscopy under 785nm laser at 100% laser power, centred at 1500cm−1, 10s exposure time and 6 accumulations acquired per sample. 1µl serum was dried and analysed with Fourier Transformed Infrared (FT-IR) Spectroscopy in transmission mode, with 64 spectral scans co-added and averaged between the spectral range of 4000cm−1- 600cm−1 with 4cm−1 resolution. Multivariate statistical analysis showed both spectroscopic techniques indicating a clear separation between subjects based on their ethnic origin. Significant clustering was seen between WE to SA and AC combined. PC-DFA loadings demonstrated vibrations and spectral features highlighting differences in metabolites of fatty acids and other lipids.

P-7 Role of Metabolomics in Age-related Macular Degeneration

PRESENTING AUTHOR: ***Deeba Husain, Mass Eye and Ear, Harvard Medical School, United States***

CO-AUTHORS: ***Ines Lains, Wonil Chung, Rachel S Kelly, John B Miller, Rufino Silva, Demetrios G Vavvas, Ivana K Kim, Jessica Lasky-Su, Liming Liang, Joan W Miller***

Purpose: Age-related macular degeneration (AMD) is the leading cause of blindness worldwide. This work aimed to assess and validate plasma metabolomic profiles of AMD and its severity stages, to better understand AMD pathogenesis. Methods: Prospective, cross-sectional study in two populations (Boston, United States and Coimbra, Portugal), including AMD subjects and controls. Fasting blood samples were analyzed using ultra-performance liquid chromatography and high-resolution mass spectrometry (Metabolon Inc). For each study site, logistic regression analysis was performed to identify metabolites linked to AMD as compared to controls (per subject model), and proportional-odds cumulative logistic regression models were built to assess metabolites varying across disease stages (both eye model). Liptak-Stouffer weighted Z-method was used for Meta-analysis. The predictive ability of the significantly identified metabolites was assessed using receiver operating characteristic (ROC) curve analysis, and their biological relevance performing pathway analysis (MetaboAnalyst). Results: We included 491 subjects; 196 (76% AMD, 24% controls) from Boston and 295 (82% AMD and 18% controls) from Coimbra. Meta-analysis revealed that, controlling for confounding factors and false discovery rate, 28 plasma metabolites differed significantly between AMD patients and controls; and 67 were significantly different across different disease stages. Pathway analysis revealed a significant enrichment of glycerophospholipids, purine, taurine and hypotaurine, and nitrogen metabolites (p 0.04). Conclusion: AMD patients present a distinct plasma metabolomic profile, that varies according to disease severity. These findings contribute to the understanding of important pathways involved in AMD, support the future development of biomarkers and may help in developing individualized treatment for this blinding condition.

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*AWARD WINNERS

BIOMEDICAL

P-8 Exercise is associated with elevated levels of 10-PAHSA and improved adipose tissue functions in elderly women

PRESENTING AUTHOR: ***Marina Oseeva, Institute of Physiology of the Czech Academy of Sciences, Czech Republic***

CO-AUTHORS: ***Marie Brezinova, Tomas Cajka, Marek Stepan, Klara Dadova, Michaela Siklova, Martin Rossmeisl, Ondrej Kuda***

The studies performed with elderly people suggest that physical activity and/or dietary interventions may improve disturbances of lipid and carbohydrate metabolism. We aimed to explore the possible synergistic effect of exercise training (ET) and supplementation of a low dose of omega-3 polyunsaturated fatty acids (Omega-3), which could enhance the beneficial metabolic effects of ET on adipose tissue (AT). Fifty non-obese elderly women (70 ± 4 years, BMI: 27 ± 4) were divided into two groups and underwent combined aerobic and resistive training (3 times a week, supervised) with or without Omega-3 supplementation (2.5 g of Calanus® oil per day) for 4 months. Before and after the interventions, insulin sensitivity was determined by hyperinsulinemic-euglycemic clamp, and samples of blood and subcutaneous AT were collected for both targeted and untargeted metabolipidomic analyses. Serum metabolomics and lipidomics revealed that Calanus oil served as a source of Omega-3, improved insulin sensitivity, but did not change the lipidomic profile of subcutaneous AT and production of pro-resolving lipid mediators. Surprisingly, levels of 10-PAHSA (palmitic acid ester of hydroxystearic acid) and several other related metabolites were strongly induced (~5-times) by the ET. PAHSA levels in serum and AT negatively correlated with AT insulin resistance parameters calculated from fasting insulin and free fatty acids levels. Our results suggests that insulin-sensitizing effects of PAHSAs might contribute to the beneficial effects of ET on the metabolism of AT in elderly women.
This project was supported by the Czech Ministry of Health 16-29182A, Czech Science Foundation GJ17-10088Y, and Calanus AS, Norway

P-9 Metabolomics of Poor Respiratory Health with Aging

PRESENTING AUTHOR: ***Haley Bayne, Brigham and Women's Hospital, United States***

CO-AUTHORS: ***Rachel S. Kelly, Avron Spiro III, David Sparrow, Pantel Vokonas, Claudia Langenberg, Isobel Stewart, Scott T. Weiss, Augusto A. Litonjua, Jessica A. Lasky-Su***

Lung function is known to decline with increasing age; however, the underlying mechanisms have yet to be fully elucidated. The aim of this study is to identify and validate metabolites associated with lung function level in adulthood utilizing two independent cohorts: the European Prospective Investigation into Cancer - Norfolk (EPIC-Norfolk) and the Normative Aging Study (NAS). We included 10,460 and 396 participants from EPIC and NAS respectively, for whom we have global metabolomic profiling with concurrent measures of lung function, including forced expiratory volume in one second (FEV1) and FEV1/forced vital capacity (FEV1/FVC). We employed multivariable regression models to identify metabolites associated with pulmonary function level, accounting for multiple testing. Nine FEV1 associated metabolites replicated in both cohorts. These included several omega-3 fatty acids: Docosahexaenoate 22:6n3 (EPIC: $\beta = 2.85$; $p = 5.37 \times 10^{-10}$, NAS: $\beta = 0.115$; $p = 0.017$); Docosapentaenoate 22:5n3 (EPIC: $\beta = 2.06$; $p = 4.84 \times 10^{-6}$, NAS: $\beta = 0.107$; $p = 0.026$); and, Eicosapentaenoate 20:5n3 (EPIC: $\beta = 1.82$; $p = 5.80 \times 10^{-5}$, NAS: $\beta = 0.090$; $p = 0.038$). These metabolites may influence pulmonary health through their anti-inflammatory effects. Two FEV1/FVC associated metabolites, both amino acids, replicated between the cohorts: cysteine-glutathione disulfide (EPIC: $\beta = -0.0077$, $p = 1.07 \times 10^{-12}$; NAS: $\beta = -0.012$, $p = 0.017$) and threonine (EPIC: $\beta = -0.00283$, $p = 0.00855$; NAS: $\beta = -0.030$, $p = 0.012$). These validated metabolites provide novel insights into the mechanisms of respiratory health.

P-10 Senescent cell metabolome reveals a novel lipid drivers and biomarkers in vitro and in vivo

PRESENTING AUTHOR: ***Arvind Ramanathan, Buck Institute, United States***

CO-AUTHORS: ***Rishi Sharma, Chris Wiley, Judith Campisi***

Cellular senescence is a multifaceted response to cellular stress or damage that results in both a permanent mitotic arrest and secretion of a number of factors with potent biological activities1-4. This senescence-associated secretory phenotype, or SASP, has largely been studied in the context of secreted proteins, many of which have been shown to have key roles in several processes including development, aging, wound healing, arthrosclerosis, cancer progression and fibrosis2,5-9. By comparison, secretion of lipids and other molecules has been understudied in the context of senescence. Here we show that senescent cells activate biosynthesis of eicosanoids – signaling lipids with potent biological effects, and this activity both promotes the inflammatory part of the SASP and reinforces mitotic arrest by a Ras-P53 axis. An finally these lipids serve as biomarkers of senolysis in vitro and in vivo.

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*AWARD WINNERS

BIOMEDICAL

P-11 The Aging Lung

PRESENTING AUTHOR: ***Charmion Cruickshank-Quinn, Agilent Technologies, United States***

CO-AUTHORS: ***Roger Powell, Irina Petrache, Nichole Reisdorph***

Lung function declines with age and increases susceptibility to a variety of lung ailments including chronic obstruction and respiratory infections in older adults. This decline has a greater effect on patients with COPD and asthma compared to healthy aging adults. To understand the effects of healthy aging on the lung environment, bronchoalveolar lavage (BAL) fluid of female C57BL/6 mice aged 3 months (young) and 12 months (old) (n=6/group) was collected from littermates housed under the same conditions. Samples underwent LC/MS untargeted metabolomics (Agilent 6220 ESI-TOF and 6560 IMMS QTOF). Spectral peaks were extracted, aligned based on accurate mass and retention time (Profinder) and missing values imputed (InfernoRDN). Compound names were assigned using an in-house library built from authentic standards using accurate mass, retention time and MS/MS or searched using exact mass and isotope ratios (MPP). T-tests were performed (Benjamini Hochberg ≤ 0.05) with fold change ≥ 1.25 (MPP). Ceramides such as Cer(d40:1) and lipid mediators such as HDHA are depleted with age. Pathway enrichment revealed non-small cell lung cancer as a perturbed pathway, where its entities DG(34:2), DG(36:3) and retinoic acid were decreased in older mice. Results suggest an age-lipid metabolism association and may signify cell membrane breakdown due to the loss of elasticity in the lungs. This study is important for understanding the effects of aging on the lung to distinguish between the normal aging process versus lung decline due to disease. Supplementation to restore the depleted compounds may help to alleviate some of the effects of aging.

P-12 Famine exposure during prenatal development has life long effect on lipid metabolism, which may in turn effect cognition

PRESENTING AUTHOR: ***Stuart Snowden, University of Cambridge, United Kingdom***

CO-AUTHORS: ***Susanne de Rooji, Aniko Korosi, Albert Koulman***

The concept that early life exposures can effect later life health is well established, and whilst differences in the lipid metabolism around the time of exposure are widely reported little work has been performed to see how lipid metabolism in later life is affected. In this study we determined if exposure to famine in utero, had a measurable effect on lipid metabolism in healthy individuals aged 60, and if these differences on the wider lipid metabolism were associated with cognitive health. Direct infusion mass spectrometry was performed on 777 human plasma samples, 448 from famine exposed individuals and 329 controls, matched for BMI etc. Random forest approaches were used to identify markers of famine exposure, as well as determine if lipid data could be used to predict cognitive performance and leptin levels. Generated models were able to predict famine exposure with a modest accuracy (64.1%) with these predictions predominantly being driven by 5 lipids, 2 cholesteryl esters and 3 phosphatidylethanolamines. Significant correlations between predicted and actual values of brain age (r = 0.529, p = 0.003) and leptin (r = 0.598, p = 3.1×10-14) were observed in models calculated using the whole of the lipid profile data, with lysophosphatidylcholines playing a crucial role in models of both brain age and leptin. In utero exposure to famine has a lasting effect on lipid metabolism with leptin potentially involved in mediating the observed metabolic reprogramming. The results also suggest a link between leptin and famine related cognitive decline via lysophosphatidylcholine metabolism.

P-13 Tandem mass spectrometry analysis of urinary methylmalonic acid as marker of metabolic vitamin B12 deficiency in older adults

PRESENTING AUTHOR: ***Michel Boutin, Universite de Sherbrooke, Canada***

CO-AUTHORS: ***Tristan Martineau, Audrey Perreault, Pierrette Gaudreau, Nancy Presse, Christiane Auray-Blais***

Vitamin B12 deficiency can lead to potentially irreversible neurological symptoms (e.g. memory deficits, gait ataxia). Up to 40% of older adults show metabolism abnormalities due to B12 deficiency. However, most present with normal levels of serum B12 so that this condition often remains under-recognized. Metabolic B12 deficiency causes an elevation of serum methylmalonic acid (MMA), an expensive test rarely available in clinical settings. Urine MMA also increases in cases of B12 deficiency, but it is unclear whether it correlates with serum MMA and can be used for diagnostic purposes. This study examined the correlation between serum and urine MMA in 35 older adults (>70 years) from the NuAge cohort with serum MMA levels varying from 165 to 15285 nmol/L. A reliable UPLC-MS/MS method for the multiplex urine analysis of MMA and creatinine was developed and validated. Briefly, 30 µL of urine was mixed with 60 µL of water containing MMA-D3 and creatinine-D3 as internal standards. A 2-minute reverse phase chromatographic method allowed the separation of MMA from succinic acid, a major isomeric interference. The Acquity I-Class UPLC system (Waters Inc.) was used. The absolute quantitation of MMA and creatinine was achieved by tandem mass spectrometry (Xevo TQ-S Micro, Waters) using the multiple reaction monitoring mode. Our results show a significant correlation between urinary MMA/creatinine ratios and MMA serum levels (Spearman r=0.59). Urine MMA measured by this UPLC-MS/MS method is promising for diagnosing B12 deficiency. This method could also be applicable as a 2nd tier-test for MMA newborn urine screening.

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*AWARD WINNERS

BIOMEDICAL

P-14 Systemic and central nervous system metabolic alterations in Alzheimer's disease

PRESENTING AUTHOR: ***Julijana Ivanisevic, University of Lausanne, Switzerland***

CO-AUTHORS: ***Vera van der Velpen, Tony Teav, Héctor Gallart-Ayala, Florence Mehl, Ioana Konz, Christopher Clark, Aikaterini Oikonomidi, Gwendoline Peyratout, Hugues Henry, Mauro Delorenzi, Julijana Ivanisevic and Julius Popp***

Metabolic alterations play an important role in Alzheimer's disease (AD) on both the systemic and central nervous system (CNS) level. To study their extent and significance in AD, quantitative metabolomics was applied to samples from clinically well-characterized AD patients. The association of the observed metabolic alterations with core pathological processes of AD was explored to understand their relation with amyloid pathology and tau-related neurodegeneration. Metabolic profiling, i.e. untargeted metabolomics and targeted quantification, was performed on paired plasma and CSF samples from clinical and biomarker-confirmed AD patients (n=40) and cognitively healthy controls without cerebral AD pathology (n=34). Targeted quantification focused on identified deregulated pathways, such as the TCA cycle and its anaplerotic pathways, as well as the neuroactive tryptophan pathway. Concentrations of several TCA cycle and beta-oxidation intermediates were higher in plasma of AD patients, whilst amino acid concentrations were significantly lower. Similar alterations were observed in CSF, which were strongly correlated with blood-brain barrier permeability. Alterations of several amino acids in CSF were associated with CSF Amyloidβ1–42. The tryptophan catabolites kynurenic acid and quinolinic acid showed significantly higher concentrations in CSF of AD patients, which, together with other tryptophan pathway intermediates, were correlated with either CSF Amyloidβ1–42, or tau and phosphorylated Tau-181. These results revealed AD-associated systemic dysregulation of nutrient sensing and oxidation and CNS-specific alterations in the neuroactive tryptophan pathway. The specific association of amino acids and tryptophan catabolites with AD CSF biomarkers suggests a close relationship with core AD pathology.

P-15 Metabolomics for the discovery of new therapeutic approach and personalized medicine: the case of exudative Age-related Macular Degeneration

PRESENTING AUTHOR: ***Pascal de Tullio, University of Liège, CIRM, Metabolomics group, Belgium***

CO-AUTHORS: ***Vincent Lambert, Matthieu Schoumacher, Julie Lecomte, Deniz Arslan, Justine Leenders, Bernadette Govaerts, Jean-Marie Rakic, Agnès Noël***

Exudative Age-related Macular Degeneration, which is characterized by a choroidal neovascularization (CNV), is the leading cause of blindness among the elderly population in developed countries. Treatment is based on intra-vitreal injection of anti-VEGF. Nevertheless, the comprehensive understanding of the pathogenesis and the evolution of this multi-factorial disease remain incomplete. Moreover, due to the long-term disease chronicity and resistance to treatment, the continuous follow-up of patients, the personalization of treatment and the discovery of new therapeutic approaches are mandatory. Because metabolomics provides a unique and direct vision to pathologies and/or treatment administration, this approach seems particularly adapted to study CNV occurrence and evolution, and to get novel and innovative insights into AMD. We applied a NMR-based metabolomics approach on sera coming from both clinical (AMD patients and controls) and pre-clinical model (a murine model that mimics CNV development). In the human cohort but also in the mice model, lactate and lipoproteins profile emerge as the main key metabolites and could be correlated to the AMD active phase and CNV development. Pharmacological normalization of lactate levels in the mice model by blocking pyruvate dehydrogenase kinase (PDK) or anti-VEGF treatments inhibits CNV formation and modified lipoproteins profiles. Mechanistically, we explore the role of lactate and we studied the modification of lipoproteins profiles according to CNV development. Altogether, we demonstrate that metabolomics is a suitable tool to deep insight into AMD and to identify some metabolites as functional, traceable and targetable molecules that open new perspectives for optimizing and personalizing treatment and patient follow-up.

P-16 Effect of calorie restriction on metabolic phenotypes of intestinal tissues in an aging model

PRESENTING AUTHOR: ***So Young Lim, Chonnam National University, South Korea***

CO-AUTHORS: ***Young-Shick Hong***

Aging accompanies the degeneration of intestinal metabolism and this intestinal dysfunction causes adverse effects to the host metabolism. Calorie restriction (CR) is an effective intervention to delay aging. However, the modification of intestinal metabolites by CR in aged model has been poorly understood. Therefore, we investigated the metabolic effect of 40% CR in various intestinal tissues and feces in an aged rat model, through 1H NMR-based metabolomics approach. The levels of fecal acetate in CR mice were significantly increased compared to control mice, indicating the alteration of the gut microbiota composition under CR condition. Moreover, the metabolic perturbation associated with nucleotide metabolism, osmoregulation, oxidative stress and bile acid metabolism were observed in the intestinal tissues of CR mice. Taken together, these metabolic features demonstrate that CR contributes to improvement of intestinal dysfunction caused by aging.

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*AWARD WINNERS

BIOMEDICAL

P-17 A Single Tissue Section Analysis Using Desi-msi and Laser Capture Microdissection for a Multimodal Cancer Research

PRESENTING AUTHOR: ***Emine Kazanc, Imperial College of London, United Kingdom***

Using clinical specimens for multiple approaches including desorption electrospray ionisation (DESI-MSI) with transcriptomic can be challenging. Downstream analysis needs more adjacent sections. Therefore, a multimodal approach in same slide would allow to co-register different types of information providing a comprehensive knowledge about molecular characterising of diseases in particularly cancer. The envisioned combination analysis raises special requirements including short DESI-MSI run time to prevent RNA degradation during analysis in room temperature. The isolated RNA must have sufficient quality to carry out qPCR, RNA seq for transcriptomics. Clinical samples were obtained from Imperial College Tissue Bank . Tissues from patient with ovarian cancer were cryosectioned at 10 µm. The DESI-MSI spectrum was collected from sections and same sections were stained with haematoxylin and eosin in RNase free conditions. Next, the regions of interest in the same slide were microdissected by Laser Capture Microdissection (Leica 7000) and RNA was isolated. RNA quality control was done by the Agilent 2100 Bioanalyzer for qPCR and Sequencing. Preliminary results showed that the RNA amount is slightly less in DESI-MSI analysed slides than non-analysed slides. Having less RNA from the DESI-MSI analysed of samples can be attributed to different reasons including analysis at room temperature causing RNA degradation. The RNA degradation of each sample was evaluated measuring the RNA Integrity Number (RIN). The results of Agilent showed that the RNA quality of samples was sufficiently good to carry out downstream analysis to identify specific genes expression, which are related with the identified metabolic profiling by DESI-MSI.

P-18 Adipocyte-induced metabolic changes in HER2-positive cancer cells: understanding breast cancer resistance to targeted therapies

PRESENTING AUTHOR: ***Manhal Mili, Institute of Analytical Sciences, France***

Targeted therapies have revolutionized the management of HER2-positive breast cancer, a particularly invasive form of cancer that responds poorly to conventional chemotherapy. However, resistance to treatment occurs in 25-60% of patients, presenting a new therapeutic challenge. Adipocytes were previously shown to act on HER2+ cells to decrease their sensitivity to targeted therapy (monoclonal antibody trastuzumab) in co-culture. Addition of a lipolysis stimulator (isoprenaline, a beta-adrenoreceptor agonist) to the system led to further decrease of HER2+ cancer cells response to trastuzumab while conversely, addition of a lipolysis inhibitor (beta-blocker propranolol) rescued the response to therapy. In order to understand the underlying mechanisms of adipocyte-modulated cancer cells resistance to trastuzumab, we present here a detailed pharmaco-metabolomic investigation of adipocyte and HER2+ cancer cell co-cultures and associated controls, conducted with or without addition of propranolol and isoprenaline. 1H 800 MHz NMR profiles were recorded to quantify metabolites present in the co-cultures supernatants and to assess the respective endometabolomes of adipocytes and HER2+ cancer cells. Quantitative analysis of HER2+ cell fingerprints (41 quantified metabolites) demonstrates substantial variations of metabolite concentrations in the presence of propranolol or isoprenaline, notably succinate and glycerol. We show that propranolol and isoprenaline, in addition to their impact on lipolysis, induced changes in adipocytes in the concentrations of UDP-glucose, 1-methylnicotinamide and several amino-acids. These results hint at a broad impact of adipocytes on HER2+ cell metabolism. Overall, our metabolomics investigation provides new insights into the mechanisms by which pharmacological modulation of lipolysis via beta-adrenoreceptors impact on HER2+ cancer cell metabolism.

P-19 NMR-based metabolomics in real-time monitoring of treatment induced toxicity in head and neck cancer and influence of individual genetic profile

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Introduction: The standard treatment for head and neck cancer (HNSCC) is radio-/chemoradiotherapy (RT/CHRT). However, it is associated with significant temporary or permanent toxic side effects in normal tissue and/or involved regions (acute radiation sequelae, ARS). In this study we investigated the real-time dynamic changes in serum metabolome during RT/CHRT in HNSCC patients to identify biomarkers of early ARS and to find their association with selected genetic variants affecting anticancer treatment response. Methods: The studied group consisted of 220 patients (22–80 years) treated with radical intent for hypopharynx, larynx, nasopharynx and oropharynx cancers. Blood serum samples were collected weekly from the day before the treatment till the RT/CHRT completion. 1H NMR spectra were acquired on a Bruker 400.13MHz spectrometer (NOESY, CPMG, diffusion edited and J-resolved sequences). Genetic polymorphisms were detected by PCR-RFLP or TaqMan probes. The data were analyzed using SIMCA (principal component analysis, partial least squares – discriminant analysis) and STATISTICA software. Results: We identified a group of outlying patients with a massive increase of signals arising from the ketone bodies (3-hydroxybutyrate, acetone, acetoacetate). Such increase correlated with the episodes of severe functional and morphological ARS. Furthermore, the NBS1 and GSTM1 polymorphisms were significantly associated with different disturbances in the serum metabolome and ARS severity (via the impact on energy metabolism and inflammation). Conclusions: Real-time molecular monitoring during RT/CHRT could be beneficial for early detection of patients with poor treatment tolerance. This work was supported by the National Science Centre (NCN), Poland, grants no. 2016/23/B/NZ5/03470 and 2015/17/B/NZ5/01387.

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BIOMEDICAL

P-20 Combined metabolite and gene expression profiling predicts biochemical recurrence in prostate cancer radiotherapy patients

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Biomarkers for prostate cancer (PCa) recurrence are highly needed for better management and follow-up of radiotherapy patients. Up-regulation of TOP2A and EZH2 have previously been connected to a more aggressive disease in radical prostatectomy (RP) patients. Further, low tissue levels of the metabolites citrate and spermine have been associated with biochemical recurrence (BCR, PSA≥0.2 ng/ml) in RP patients. The main aim of the present study was to investigate the potential of gene expression and MR tissue metabolites as biomarkers for BCR in transrectal ultrasound-guided (TRUS) biopsies of PCa radiotherapy patients prior to treatment (n=172 samples). TOP2A and EZH2 expression were assessed using immunohistochemistry, and high-resolution magic angle spinning MR spectroscopy was performed to measure metabolites in the same TRUS biopsy. In cancer patient samples without hormone treatment, concurrent up-regulation of TOP2A and EZH2 were identified as predictors of BCR among PCa radiotherapy patients (n=31, p=0.0079). Lower levels of citrate (p=0.0037) and polyamines (p=0.0057) prove the same. TOP2A and EZH2 in combination with significantly lower levels of either citrate or polyamines in cancer samples was confirmed as potential biomarkers for BCR among radiotherapy patients in TRUS biopsies collected prior to treatment (n=6, p=0.00027, p<0.0001 respectively). In conclusion, combination of TOP2A and EZH2, MR metabolites citrate and polyamines are suggested as biomarkers for BCR in PCa radiotherapy patients not receiving neoadjuvant hormonal treatment measured in TRUS biopsies prior to treatment; however, this needs validation in a larger cohort. This study offers a translational potential detecting metabolites using in vivo MR spectroscopic imaging.

P-21 Urinary GC-MS untargeted metabolomics for prostate cancer diagnosis. Preliminary results

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CO-AUTHORS: *Alessandra Biancolillo, Rasmus Bro, Francesco Porpiglia, Alberto Salomone, Marco Vincenti*

Prostate carcinoma is the principal cause of cancer-related death in men. The prostate specific antigen (PSA) dosage is, at the present, the unique clinical test available to screen for this pathology. PSA is highly sensitive but not enough specific for malignancy, providing a significant amount of misleading results. Thus, new biomarkers which could improve the diagnostic power of prostate screenings are needed. The present work is a preliminary study aimed to discover new urinary biomarkers suitable for this purpose. Ninety subjects, divided into controls and prostate carcinoma-affected, were recruited. Two aliquots of each sample were treated as follows: proteins were precipitated, two subsequent extractions (at acid and basic conditions) were performed for each aliquot and the dried extracts were derivatized using trifluoroacetic anhydride (aliquot A) and trimethylsilyl derivatizing mixture (aliquot B). The full-SCAN GC-MS spectra (two for each sample) were then processed with the following chemometric approach: (i) the alignment was performed by double warping algorithm; (ii) The spectral deconvolution was carried out using the PARADISe software, based on the PARAFAC2 algorithm; (iii) The semiquantitative report provided by the software was then subjected to two different and independent variable selection procedures: (a) Variable Importance Projection and (b) Covariance Selection algorithm; (iv) The reduced datasets were used to build two classification models, which performances were compared. The validation of the models was performed using a repeated double cross validation approach, and the obtained preliminary results are promising, scoring near to 90% for both specificity and sensitivity.

P-22 Novel metabolomic predictors of incident colorectal cancer in men and women

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CO-AUTHORS: *Amit D. Joshi, Brendan J. Guercio, Oana A. Zeleznik, Mingyang Song, Fred K. Tabung, A. Heather Eliassen, Kana Wu, Charles S. Fuchs, Edward Giovannucci, Jeffrey A. Meyerhardt, Andrew T. Chan*

Background: Metabolomic profiles reflect the combined influence of genetic and lifestyle factors in the development of colorectal cancer (CRC). To date, few prospective studies have conducted metabolome-wide association analyses of pre-diagnostic plasma in CRC risk. Methods: We conducted a nested case-control study among 32,786 women (Nurses' Health Study [NHS]) and 18,159 men (Health Professionals Follow-up Study [HPFS]) who provided blood (~60% fasting) between 1989-95. Over a 17-year follow-up period, we documented 665 (409 in NHS, 256 in HPFS) incident CRC cases which were matched 1:1 to 665 controls based on cohort, age, race, time of collection, and fasting status. We conducted metabolomic profiling using liquid chromatography-mass spectrometry to identify 530 known plasma metabolites. Weighted correlation network analysis (WGCNA) was done to obtain modules of interconnected molecules with high topological overlap. Logistic regression was used for association analyses, and multiple comparisons correction was performed using α-threshold P<8.5x10-4. Results: We observed significant associations with CRC risk for 5 metabolites, including C58:7-TAG (OR=0.81,P=6.6x10-4) in all participants, myristoleic acid (OR=1.32,P=4.3x10-4) in women, and N2,N2-dimethylguanosine (OR=1.61,P=4.8x10-4) in men. Associations were stronger when restricted to fasting samples. Among WGCNA-derived modules, association differed according to sex, with nucleic acid module associated with CRC in men (P=0.010), and carnitine module associated in all participants (P=0.013). Conclusions: We identified prediagnostic metabolites associated with CRC in 3 groups, acylglcyerols, purines, and ω-5-fatty acids, with distinct profiles in men compared with women. These results provide insight into the etiopathogenesis of CRC and have implications for risk prediction and novel prevention strategies.

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P-23 Evaluation of urinary volatile metabolites as potential biomarkers for prostate cancer diagnosis

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Prostate cancer (PCa) is the second most common malignancy in men. Prostate specific antigen (PSA) is the most frequently used biomarker for PCa screening but, due to its recognized limitations, nowadays, U.S. Preventive Services Task Force recommends against PSA screening. The analysis of volatile compounds emanating from biological samples is a major promising approach for finding new effective diagnostic markers for PCa. The purpose of this work was to study the urinary volatile metabolic profile of patients with PCa (n=40) and non-cancer controls (n=42) with the aim of identifying a potential urinary volatile pattern as a non-invasive strategy to detect PCa. A metabolomics approach based on headspace solid-phase microextraction gas chromatography–mass spectrometry was performed to investigate volatile organic compounds (VOCs) in general and, more specifically, volatile carbonyl compounds (VCCs) present in urine samples. Considering both approaches, a panel of 30 volatile compounds descriptive of PCa was defined, capable of discriminating PCa patients from controls. Furthermore, an external validation set (n=18 PCa and n=18 non-cancer controls) was used to calculate the sensitivity, specificity and accuracy of the defined panel. Our results revealed that this panel of 30-volatiles was able to perfectly predict PCa, with 100% sensitivity, specificity and accuracy. Overall, our results disclose a biomarker panel that has the potential to be used in clinic for PCa diagnosis. A.R.L thanks FCT, Portugal, for her PhD grant (SFRH/BD/123012/2016). This work was financed by national funds from FCT/MEC (UID/Multi/04378/2013) and co-financed by the ERDF under the PT2020 Partnership Agreement (POCI/01/0145/FEDER/007728).

P-24 Associations between metabolites and pancreatic adenocarcinoma risk in a large prospective study

PRESENTING AUTHOR: ***Rachael Stolzenberg-Solomon, DCEG, NCI, NIH, United States***

CO-AUTHORS: ***Andriy Derkach, Steve Moore, Stephanie Weinstein, Demetrius Albanes, Joshua Sampson***

Background: Metabolomics profiles may offer improved insights into etiology and the system of factors involved in the process of pancreatic adenocarcinoma (PDAC) tumorigenesis. Methods: We conducted an untargeted analysis of 554 known metabolites measured in pre-diagnostic serum (up to 24 years) to determine their association with incident PDAC in a nested-case control study of male smokers (373 matched case-control sets) with replication in an independent nested-case control study in that included women and non-smokers (107 matched sets). Controls were matched to cases by age, sex, race, date of blood draw and follow-up time. We used conditional logistic regression to calculate odds ratios (OR) and 95% confidence intervals (CI) for a 1 standard deviation increase in log-metabolite level separately in each cohort and combined the two ORs using a fixed effects meta-analysis. Results: Thirty-one metabolites were significantly associated with PDAC at an FDR < 0.05 with 12 metabolites below the Bonferroni-corrected threshold (P-value < 9.04 x 10-5). Similar associations were observed in both cohorts. The dipeptides glycylvaline, aspartylphenylalanine, pyroglutamylglycine, phenylalanylphenylalanine, phenylalanylleucine, and tryptophylglutamate and amino acids aspartate and glutamate were positively while the dipeptides tyrosylglutamine and α-glutamyltyrosine, fibrinogen cleavage peptide DSGEGDFXAEGGGVR, and glutathione related amino acid cysteine-glutathione disulfide were inversely associated with PDAC after Bonferroni correction. Five top metabolites demonstrated significant time varying associations (P-value < 0.023) with associations becoming attenuated 10-15 years after participants' blood collection. Conclusion: Our results demonstrate pre-diagnostic metabolites related to subclinical disease, glutathione metabolism, and adiposity/insulin resistance are associated with PDAC.

P-25 Exploratory metabolomics of urine and plasma to identify novel pharmacodynamic biomarkers in a phase I clinical trial of AZD3965

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A key metabolic alteration in tumour cells is increased dependency on glycolysis, resulting in the production of lactate which is transported out of cells by monocarboxylate transporters (MCT1 & MCT4) which are therefore a therapeutic target in cancer. Current literature suggests that inhibition of MCT1 in preclinical models can constrain cancer cell growth in tumours with low MCT4 expression. To date the systemic pharmacodynamic effects of the small-molecule non-competitive inhibitor of MCT1, AZD3965, the agent of study in this first-in-class (FIC) trial CRUKD12/004, have not been fully characterised. Preclinical metabolomics studies conducted at Imperial College London indicated that AZD3965 exposure caused increases in lactate, ketone bodies (also MCT1 substrates) and citrate in blood plasma and urine independently of tumour burden and tumour expression of MCT1, and also caused decreases in fatty acids in blood plasma. We used NMR spectroscopy of urine and plasma samples from 34 patients from the trial to specifically monitor lactate and other ketone bodies and in addition a metabolomics screen using a well-validated LC-MS/MS protocol (Biocrates AbsoluteIDQ p180 kit) on plasma. Metabolomics profile of plasma and urine appears to reflect response of AZD3965 treatment, and especially total urinary excretion of lactate and ketone bodies offers proof of target engagement. This effect is not mirrored in plasma suggesting that this may be primarily a renal effect. Observed systemic metabolic effects of AZD3965 exposure appear to lessen with repeated dosing, suggesting a rapid adaptive response. Metabolomics profile can offer insides in understanding the mechanism of drug toxicity.

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BIOMEDICAL

P-26 Preoperative Metabolomic Signature of Prostate Cancer Recurrence.

PRESENTING AUTHOR: ***David Gaul, Georgia Institute of Technology, United States***

CO-AUTHORS: ***Chaevien S. Clendinen, Rebecca S. Arnold, John A. Petros, Arthur S. Edison, Facundo M. Fernández***

Up to 50% of prostate cancer surgical patients will suffer from biochemical recurrence manifested by detectable serum prostate specific antigen levels even after prostate removal. Relatively limited pre-operative information has not yielded adequate prognosis to guide a patient's treatment decision. More clinical data is needed to improve prognosis. New biomarkers can arise from metabolome analysis of patient serum to aid physicians and patients in developing a treatment plan. We applied a multiplatform (NMR + LC–MS) metabolomics approach to the study of preoperative metabolic alterations associated with prostate cancer recurrence. Correlation analysis on the serum metabolite abundances from prostate cancer patients (n = 40 remission vs n = 40 recurrence) showed significant alterations in several pathways, including amino acid metabolism, purine and pyrimidine synthesis, tricarboxylic acid cycle, tryptophan catabolism, glucose, and lactate. In addition, higher lipid abundances were observed in the serum from recurrent patients for several classes that included triglycerides, lysophosphatidylcholines, phosphatidylethanolamines, phosphatidylinositols, diglycerides, acyl carnitines, and ceramides. Machine learning approaches led to the selection of a 20-metabolite panel from a single preoperative blood sample that enabled prediction of recurrence with 92.6% accuracy, 94.4% sensitivity, and 91.9% specificity under cross-validation conditions.

P-27 A Metabolomics-based Adaptive Strategy for Monitoring and Delivering Systemic Therapy

PRESENTING AUTHOR: ***Jodi Rattner, University of Calgary, Canada***

CO-AUTHORS: ***Chris O'Callaghan, Dongsheng Tu, Patricia A. Tang, Lillian L. Siu, Jeremy Shapiro, Karen Kopciuk, Hans J. Vogel, Oliver F. Bathe***

Background. Chemotherapy options for treating colorectal cancer (CRC) have expanded in recent years. Treatment benefit is assessed in a costly and untimely manner, typically by serial CT scans beginning 2 – 3 months after starting drug. Our aim was to develop a blood-based biomarker that would identify patients who are not responding to chemotherapy, soon after chemotherapy is initiated. Methods. Samples and linked clinical data of metastatic colorectal cancer patients were obtained from a large clinical trial (NCIC-CTG CO.20)Response to chemotherapy was determined by RECIST 1.1 criteria. Serial plasma metabolomic profiles from a discovery set of patients(n=68), treated with cetuximab or cetuximab+brivanib, and disease-free controls(n=48) were analysed using gas chromatography-mass spectrometry (GC-MS). A response model for CRC was derived using orthogonal partial least squares-discriminant analysis (O-PLS-DA) based on treatment-related changes in metabolites. Metabolomic models suitable for distinguishing response categories and progression of disease were generated. Results. Using GC-MS, 386 compounds were detected. By as early as 4 weeks after chemotherapy was initiated, treatment-related changes that discriminated measurable response, stable disease and progression could be detected. The model that signaled disease progression (which marks treatment futility) was robust (R2Y=0.822; Q2Y=0.605; P-Value=<0.0001). This biomarker consisted of 25 metabolites, including important intermediates involved in nucleotide synthesis and energy metabolism. Conclusions. Treatment-related changes in circulating metabolites appear shortly after initiating chemotherapy. The pattern of changes varies with the type of treatment response. Progressive disease has a particularly distinct set of changes. External validation will be required to further assess these biomarkers.

P-28 Untargeted metabolomics of archived dried blood spots reveals evidence of maternal and neonatal nutrition as risk factors for childhood leukemia

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Early-life exposures are believed to play an important etiologic role in the development of pediatric acute lymphoblastic leukemia (ALL). Yet direct analysis of exposures have not been undertaken to date, primarily due to technological barriers. Archived neonatal blood spots (NBS) collected within the first days of life offer a means to investigate small molecules that reflect early-life exposures and biological response that pre-date the onset of clinical disease. We obtained NBS for 334 ALL cases and 324 healthy matched controls as part of the California Childhood Leukemia Study. Untargeted metabolomics was performed on the extracts of NBS punches (4.7-mm, ~ 8 µL of blood), with an Agilent 1290 UHPLC system connected to a 6550 QTOF HRMS (Santa Clara, USA) in ESI (-) mode. Subjects were stratified by early (1-5 y) and late (6-14 y) diagnosis, and an ensemble of feature-selection methods used to pinpoint metabolites predictive of case status. Covariates representing suspected risk factors for ALL were also evaluated. Mutually-exclusive sets of lipids and fatty acids were associated with ALL phenotypes, including 9 and 19 metabolites in the early- and late-diagnosis groups, respectively. In the late-diagnosis group, a prominent cluster of metabolites contained molecules with 18:2 fatty-acid chains, suggesting that newborn exposure to the essential nutrient, linoleic acid, increased ALL risk. Interestingly, abundances of these 18:2 lipids were greater in infants who were fed formula rather than breast milk (colostrum) and increased with the mother's pre-pregnancy body mass index, suggesting possible etiologic roles of newborn nutrition.

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P-29 Relevance of (R,R')-4'-methoxy-1-naphthylfenoterol for treatment against metastatic colorectal cancer

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CO-AUTHORS: *Danuta Dudzik1,2, Irving W. Wainer3, Wiktoria Struck-Lewicka1, Małgorzata Waszczuk-Jankowska1, Michał J. Markuszewski1, Michel Bernier4, Danuta Siluk1, 1 Department of Biopharmaceutics and Pharmacodynamics, Faculty of Pharmacy, Medical University of Gdańsk, Poland, 2 Center for Metabolomics and Bioanalysis (CEMBIO), Faculty of Pharmacy, San Pablo CEU University, Madrid, Spain, 3 PAZ Pharmaceuticals, Washington, DC, USA, 4 National Institute on Aging, National Institutes of Health, Baltimore, Maryland, USA*

Metastatic colorectal cancer (CRC) is the third leading cause of cancer-related death worldwide. The clinical management is challenging due to CRC innate and/or acquired multidrug resistance to standard and targeted chemotherapies as well as resistance to immuno-oncology approaches. We studied the anticancer effects of 4'-methoxy-1-naphthylfenoterol (MNF) acting through the inhibition of G protein-coupled receptor GPR55 and activation of the β2-adrenergic receptor. We provide the results from untargeted metabolomics analysis of plasma and tumor tissues obtained from (R,R')-MNF-treated and control animals, designed to explore the biological mechanisms associated with the observed antitumor response and to identify potential biomarkers of these effects. Female BALB/c mice with a subcutaneous CT26 CRC tumor were divided into three experimental groups: (R,R')-MNF at 20mg/kg (n=9) or 30mg/kg (n=8), and a vehicle-treated (n=10). The changes in plasma and tumor metabolomic profiles associated with the MNF action were evaluated by liquid chromatography mass spectrometry (RP-HPLC-ESI-TOF-MS) analysis. Raw data acquired was pre-processed, filtered, and corrected for signal drift. A combination of univariate and multivariate statistical analyses was used to evaluate the drug effect on CRC. Metabolite annotation was accomplished by searching against several databases including HMDB, KEGG, Metlin, and LipidMaps. Among the most differentiated metabolites whose levels were significantly impacted by MNF were those belonging to the lipid metabolism pathway, including glycerolipids, glycerophospholipids, and sterol lipids. These results are consistent with our previous data reporting MNF activity in pancreatic cancer. The knowledge generated in this study has advanced our understanding of the anticancer molecular mechanisms of MNF.

P-30 The discovery of potential cancer biomarkers in human plasma using GC- and GCxGC-TOFMS

PRESENTING AUTHOR: *Ralf Loescher, LECO European Application and Technology Center, Germany*

CO-AUTHORS: *David E. Alonso, Joe Binkley, Habtom Ressom, Cristina Di Poto*

High mortality rates exist for individuals with hepatocellular carcinoma. There is a critical need for discovery of early stage HCC biomarkers to elicit rapid and effective treatments. GC-MS based metabolomics is an ideal approach for investigating cancer physiology for discovery of liver disease diagnostic markers. The objective of this research study was to implement an untargeted, multiplatform, analytical approach for identification of candidate HCC biomarkers in humans using comprehensive GC- and GCxGC-TOFMS. After sample preparation the dry samples were derivatized in two-steps: 1) Methoximation and 2) silylation. Derivatized samples and quality control standards were injected into the chromatograph and separation performed using two different polarity columns. Mass spectra were collected using a mass range of 50 to 510 and an acquisition rate of 10 spectra per second (200 sps for GCxGC). Data were processed using untargeted Peak Find and compounds were characterized through retention index filtering, similarity searches and formula determinations. Analyses of plasma samples resulted in composition maps displaying a wide variety of compounds including acids, diacids, amino acids, bases, fatty acids, a large assortment of simple sugars, and reduced/oxidized sugars. Transitioning from GC-TOFMS to high performance GCxGC-TOFMS resulted in a greater than 2-fold increase in characterized compounds. Statistical processing of the data led to the identification of potential candidate markers for HCC disease including organic acids, amino acids and reduced sugars.

P-31 Volatile organic compounds as biomarkers of prognosis in palliative lung cancer patients

PRESENTING AUTHOR: *Elinor Chapman, Palliative Care Institute Liverpool, University of Liverpool, United Kingdom*

CO-AUTHORS: *David Hughes, John Ellershaw, Mark Boyd, Chris Probert, Seamus Coyle*

Diagnosing dying and predicting prognosis for palliative patients is an ongoing problem for physicians as there are few good biomarkers (Reid, VL et al., PLoS One, 2017). Having more certainty about prognosis is crucial for patients, their families and clinicians to provide the best care possible. We hypothesise there is a metabolic process to dying that could yield quantifiable biomarkers. A feasibility study showed that volatile organic compounds (VOCs) in urine changed in the last days and weeks of life in a small mixed cancer group (Coyle, S. et al., BMJ Open, 2016). In this pilot study: 424 urine samples were collected from 162 patients with advanced lung cancer from six sites across Merseyside. 63 patients provided a sample in the last month (28 days) of life and 29 patients in the last week of life. Urinary VOCs were examined by gas chromatography-mass spectrometry (GC-MS). Least Absolute Shrinkage and Selection Operator (LASSO) logistic regression was used to analyse data and create models. A model predicting whether an individual would die within three days based on only two VOCs, generated an AUC = 0.80 (0.68-0.93), was 93% specific and 68% sensitive. Other models predicting between 4-10 days of life or 11-17 days of life remaining were generated. Urine analysis could be used to predict the dying process and lead to the identification of prognostic biomarkers. Future work will focus on optimisation of urinary VOC analysis, refinement of models and verifying in a validation cohort of patients with lung and other cancers.

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P-32 Metabolic alterations in patients with cancer: searching for metabolomic biomarkers of breast cancer

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CO-AUTHORS: ***David Rojo Blanco, Marija Kirzic, Natalija Dedic Pavletic, Kamelija Zarkovic, Damir Vrbanec, Biserka Orehovec, Coral Barbas, Neven Zarkovic***

Breast cancer (BC) is a heterogeneous disease and one of the major causes of cancer death in women. One of the main hallmarks of cancer is the ability of cancer cells to alter their metabolic phenotype, which allows them to survive and proliferate against all odds. Therefore, the aim of this study was to apply LC-MS based untargeted metabolomics approach, as previously described [1], in order to identify new metabolomic biomarkers associated with BC pathology. Study included plasma samples from BC patients prior to any treatment (N=41) and matching controls (N=30). Fifty-seven chemical compounds were found to be significantly altered in BC patients. The resulting list of statistically significant accurate masses was annotated using the CEU Mass Mediator search tool (http://ceumass.eps.uspceu.es/mediator/), and the biological role of suggested compounds was additionally evaluated to exclude the less probable identification matches. Our results indicate a significant increase in the expression of different fatty acyls, including several straight chain, branched and unsaturated fatty acids, docosanoids, eicosanoids, octadecanoids, fatty aldehydes and amides. Increased levels of certain monoacylglycerols and different glycerophospholipids were also found to be characteristic for BC subjects. We also detected alteration of amino acid and bilirubin metabolism in BC patients and differential expression of C19 and C20 steroids. These findings suggest a key role of lipid classes and molecular species in BC growth and development and possibly lead us a step closer to new metabolomic biomarkers in BC.

P-33 Investigating the drug responsiveness on the metabolic pathways in PDAC-PDX models

PRESENTING AUTHOR: ***Adja Zoumaro-Djayoon, Leiden University, Netherlands***

CO-AUTHORS: ***Diana Behrens, Amy Harms, Thomas Hankemeier***

Despite all the progresses made in the identification of molecular alterations related to pancreatic ductal adenocarcinoma cancer (PDAC), effective treatment is still lacking, to reduce the high mortality rate in this cancer. To move forward in pancreatic cancer research, new concepts and methods are required, to find better drug targets and early diagnostics markers. We used metabolomics to investigate the effects of the anti-cancer drugs gemcitabine, abraxane (Nab-paclitaxel) and erlotinib on the metabolism of newly developed patient derived xenografts (PDX). For this purpose, targeted metabolomics platforms that cover metabolites of major metabolic pathways including glycolysis, central metabolism, lipids, amino acids, and signalling lipids (inflammatory mediators), were applied to treated and non-treated tumor tissues from patient derived xenografts of two patients. The comparison between the metabolomics profiles of treated and untreated models, revealed changes in the metabolic profiles. In xenografts derived from one patient, abraxane treatment significantly decreased the levels of amino acids and increased the levels of lipid metabolites, while an increase in the level of sphingomyelins was observed with gemcitabine treatment. However, no significant changes were observed in the metabolic profiles of the second patient upon treatment. Our findings can contribute to the understanding of oncogenic mechanisms in pancreatic ductal adenocarcinoma cancer as well as improve the monitoring of treatments in this cancer.

P-34 The Effects of Omega-3 Supplementation on the Brain Lipidome after Chemotherapy

PRESENTING AUTHOR: ***Rachel Kopec, The Ohio State University, United States***

CO-AUTHORS: ***Djawed Bennouna, Melissa Solano, Tonya S. Orchard, Maryam B. Lustberg, A. Courtney DeVries***

Breast cancer chemotherapy agents negatively affect long-term brain functioning in some breast cancer survivors. Neuroinflammattory damage may play an important role in changing brain structure or altering signaling, resulting in cognitive impairment. We hypothesized that a diet enriched with long chain omega-3 polyunsaturated fatty acids (n-3 PUFA), would result in a greater inflammation resolving response mediated by specialized pro-resolving mediators (SPMs i.e. omega-3 derived metabolites which attenuate inflammation), and reduce oxidation of hippocampal brain lipids. To determine how n-3 PUFA supplementation may confer protection after chemotherapy, ovariectomized, C57BL/6 mice were fed a diet with or without 2% n-3 PUFA enrichment for 4-6 weeks, followed by a two-injection chemotherapy or vehicle regimen. In study 1, animals (n=120) were sacrificed at 4, 7, and 14 days after the last injection, and targeted HPLC-MS/MS analyses quantitated specialized pro-resolving mediators (SPMs) in whole brain. In study 2, animals (n=80) were sacrificed 7 days after the last injection, and untargeted LC-HRMS lipidomics were performed on the hippocampus. Study 1: SPM resolvin D1 was quantifiable in all samples regardless of treatment or dietary group, but no differences were observed. Study 2: PLS-DA modeling revealed ~500 metabolites driving significant separation between the n-3 PUFA supplemented vs. non-supplemented animals (R2=0.67, Q2=0.64, P-value= 0.014), and that this dietary effect was greater than the effect of chemotherapy 7-days post-chemotherapy treatment. Identification of distinguishing lipids is ongoing. Taken together, the results of studies 1 and 2 suggest that other, non-inflammatory pathways are likely involved in driving this separation between lipids.

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*AWARD WINNERS

BIOMEDICAL

P-35 Urine-NMR metabolomics for screening of advanced colorectal adenoma and early stage colorectal cancer

PRESENTING AUTHOR: ***Hyuk Nam Kwon, University of Helsinki, Finland***

CO-AUTHORS: ***Eun Ran Kim, Hoonsik Nam, Jae J. Kim, Sunghyouk park, Young-Ho Kim***

Although colorectal cancer (CRC) is considered one of the most preventable cancers, no non-invasive, accurate diagnostic tool to screen CRC exists. We explored the potential of urine nuclear magnetic resonance (NMR) metabolomics as a diagnostic tool for early detection of CRC, focusing on advanced adenoma and stage 0 CRC. Urine metabolomics profiles from patients with colorectal neoplasia (CRN; 36 advanced adenomas and 56 CRCs at various stages, n = 92) and healthy controls (normal, n = 156) were analyzed by NMR spectroscopy. Healthy and CRN groups were statistically discriminated using orthogonal projections to latent structure discriminant analysis (opLs-DA). the class prediction model was validated by three-fold cross-validation. The advanced adenoma and stage 0 CRC were grouped together as pre-invasive CRN. The OPLS-DA score plot showed statistically significant discrimination between pre-invasive CRN as well as advanced CRC and healthy controls with a Q2 value of 0.746. In the prediction validation study, the sensitivity and specificity for diagnosing pre-invasive CRN were 96.2% and 95%, respectively. The grades predicted by the OPLS-DA model showed that the areas under the curve were 0.823 for taurine, 0.783 for alanine, and 0.842 for 3-aminoisobutyrate. In multiple receiver operating characteristics curve analyses, taurine, alanine, and 3-aminoisobutyrate were good discriminators for CRC patients. NMR-based urine metabolomics profiles significantly and accurately discriminate patients with pre-invasive CRN as well as advanced CRC from healthy individuals. Urine-NMR metabolomics has potential as a screening tool for accurate diagnosis of pre-invasive CRN.

P-36 Insight the metabolic avidity in the KRAS mutated non-small cell lung cancer cells by flux analysis and biochemical modelling

PRESENTING AUTHOR: ***Laura Brunelli, Istituto Ricerche Farmacologiche Mario Negri IRCCS, Italy***

CO-AUTHORS: ***Ewelina Węglarz-Tomczak, Elisa Caiola, Mirko Marabese, Giovanna Sestito, Massimo Broggini, Hans Westerhoff, Roberta Pastorelli***

Non–small-cell lung cancer (NSCLC) is a heterogeneous disease, with multiple different oncogenic driver mutations. Currently, there are no successful treatment targeting NSCLC KRAS-mutated patients. In NSCLC roughly half of the tumors with activating KRAS genetic lesions also have serine/threonine kinase 11 (LKB1) deletions or inactivating mutations. The presence of LKB1 loss in KRAS-mutant tumor may represent a significant source of heterogeneity and contribute to the worst response to therapy. Since both KRAS and LKB1 lesions have impact on cellular metabolism, it is fundamental to discern the metabolic effects induced by single and concomitant genetic events, in view of the potential exploitation of such metabolic dependencies for novel therapeutic interventions. Using 13C-metabolic flux analysis, we demonstrated how isogenic NSCLC cells harbouring both KRAS/LKB1 alterations were able to exploit the central cellular metabolic routes through a heightened metabolite production compare to the single ones. Further, to recognize such peculiar metabolic behaviour we performed in silico modelling on genome wide metabolic reconstruction. Based on RNA-seq, metabolic enzymes abundance and composition of the medium and using flux balance analyses we predicted metabolic changes. The observed differences in metabolites production were correlated with experimental observations supporting that the enhanced metabolic activity of cells with both genetic lesions rendered the viability of these cells more susceptible to energetic stress such as glycolysis inhibition and/or nutrients limitation than those harboring single lesions. This observation raises the prospect that energy stress may affect NSCLC cells harbouring co-occurring lesions, which may render them more susceptible to cytotoxic drugs.

P-37 Comprehensive evaluation of a one-step sample preparation for global LC-MS lipidomics of cancer cell cultures

PRESENTING AUTHOR: ***Gillian Mackay, CRUK Beatson Institute, United Kingdom***

CO-AUTHORS: ***Giovanny Rodriguez-Blanco, Victor H Villar, David Sumpton***

Lipidomics aims to provide a global analysis of all lipid species present in biological samples. Lipids play an important role in cell membrane integrity, energy storage, and cell signalling, all of which are relevant in cancer development and progression. Standard methods in lipidomics profiling of cell cultures involve biphasic separation using organic solvents such as chloroform or methyl tert-butyl ether to extract the lipids, followed by evaporation and reconstitution in an LC-MS compatible solvent. Some technical issues can arise using this biphasic extraction and reconstitution: we experienced issues with reproducibility between biological replicates and inaccuracy in our normalisation to protein content, an important parameter when comparing cells growing at different rates. In this study, we propose a rapid single step procedure involving simultaneous lipid extraction and protein precipitation from cell culture plates using either isopropanol (IPA) or a mixture of 1:1 butanol/methanol (BuMe). To evaluate the extraction efficiency of IPA and BuMe compared with chloroform/methanol, HepG2 (liver cancer) cells were grown in a standard medium, in a lipid-rich medium and after treatment with a DGAT inhibitor, inhibiting triacylglycerol biosynthesis. Our LC method and mass spectrometry parameters were addressed, as were various methods and software for data analysis. We compared global changes in lipid classes, differences in lipid intensities and the effect of the DGAT inhibitor, with the three different extraction procedures.

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*AWARD WINNERS

BIOMEDICAL

P-38 Investigation of mevalonate pathway by quantitative metabolomics and isotopologue profiling

PRESENTING AUTHOR: ***Floriant Bellvert, MetaToul-MetaboHUB, France***

CO-AUTHORS: ***Hanna Kulyk-Barbier, Sara Castano-Cerezo, Pierre Millard1, Jean-Charles Portais, Stéphanie Heux, Gilles Truan, Floriant Bellvert.***

Mevalonate pathway is an important metabolic pathway which plays a key role in multiple cellular processes. In recent years the mevalonate pathway has become a challenging and, in the meantime, fascinating topic, when a large number of experimental and clinical studies suggested that inhibition of nonsterol isoprenoids might have valuable interest in human pathology. These molecules that are essential for cell growth and differentiation appear to be potential interesting therapeutic targets for many areas of ongoing research: oncology, autoimmune disorders, atherosclerosis, and Alzheimer disease. Thus, functional understanding of their biosynthesis is important key. However, available methods do not yet allow accurate quantification and tracing of stable isotopes incorporation for all the isoprenoids precursors (mevalonate intermediates and prenyl pyrophosphates). We present a complete methodology for functional analysis of isoprenoids biosynthesis, with a novel quantification method based on liquid chromatography coupled to high-resolution mass spectrometry. This workflow covers all the experimental and computational steps from sample collection and preparation to data acquisition and processing. It ensures accurate, absolute quantification (RSD < 20 %) of all mevalonate and prenyl pyrophosphates intermediates with a high sensitivity over a large linear range (from 0.1 to 50 pmol). Determination of their isotopologue distributions in isotopic labeling experiments was performed, opening the way for 13C-metabolic flux analysis of isoprenoids biosynthesis. In conclusion, the described methodology fills one of the last technical gaps for functional studies of isoprenoids biosynthesis and should be applicable to other eukaryotic and prokaryotic (micro)organisms after adaptation of some organism-dependent steps.

P-39 Prospective Serum Metabolomic Profile of Prostate Cancer Risk in African-Americans from Two U.S. Cohorts

PRESENTING AUTHOR: ***Demetrius Albanes, U.S. National Cancer Institute, United States***

CO-AUTHORS: ***Jiaqi Huang, Stephanie Weinstein, Joshua Sampson, Christopher Haiman, Loic Le Marchand, Tracy Layne***

African-American men are under-studied in prostate cancer research despite having substantially higher disease incidence compared to other races. Recent prospective studies conducted primarily among men of European ancestry identified several plasma/serum metabolites associated with prostate cancer risk, including lipids (fatty acids, acylcarnitines, sphingomyelins, inositols, glycerphospholipids, and androgens), dipeptides and gamma-glutamyl amino acids, nucleotides, TCA cycle compounds and antioxidants. We examined the prospective serum metabolomic profile of prostate cancer risk in African-Americans in order to elucidate possible biological mechanisms underlying their greater disease burden. We meta-analyzed two case-control studies nested within the Multiethnic Cohort and Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial cohort (592 cases/592 controls, matched on age, study center, and blood collection date). Samples were analyzed using ultrahigh performance liquid chromatography/tandem mass spectrometry (Metabolon, Inc.) that identified 997 known compounds. Conditional logistic regression estimated ORs and 95% CIs for 1-s.d. increments in metabolite levels that were meta-analyzed using a fixed-effects model. Median time from serum collection to diagnosis was 5 years (maximum 19 years). Metabolites showing the strongest associations with prostate cancer risk (FDR q-value=0.2) were p-cresol glucuronide (1-s.d. OR 0.80, p=0.00045), hypotaurine (1-s.d. OR 1.25, p=0.0006), p-cresol sulfate (1-s.d. OR 0.81, p=0.00078), indolin-2-one (1-s.d. OR 0.81, p=0.00084), asparagine (1-s.d. OR 1.23, p=0.001), indole-3-carboxylic acid (1-s.d. OR 0.80, p=0.002), and taurine (1-s.d. OR 1.21, p=0.002). The metabolomic profile of prostate cancer risk in this investigation of black men suggests an etiologic role for microbiota-related metabolites that differs substantially from metabolites previously identified in populations of European ancestry.

P-40 Radiometabolomics – correlation of metabolomics and PET/MRI imaging data of non-small cell lung cancer patients

PRESENTING AUTHOR: ***Michal Ciborowski, Medical University of Bialystok, Poland***

CO-AUTHORS: ***Joanna Kisluk, Tomasz Kowalczyk, Ewa Sierko, Joanna Reszec, Malgorzata Mojsak, Karolina Pietrowska, Joanna Godzien, Adam Kretowski, Jacek Niklinski***

Lung cancer (LC) remains the leading cause of cancer death worldwide with non-small cell lung cancer (NSCLC) constituting about 85% of all LC cases. Recently positron emission tomography/magnetic resonance imaging (PET/MRI) is actively used to study LC. Measurement of 2-deoxy-2-[18F]-fluoro-D-glucose uptake by tumor reflects its increased rate of aerobic glycolysis. Among others, PET/MRI provides information about tumor size (Metabolic Tumor Volume, MTV) and metabolic activity (Standardized Uptake Value, SUV). Correlation of PET/MRI results with metabolomics data may help to indicate metabolic pathways engaged in tumor growth and activity. Plasma samples of 47 NSCLC patients (65±6 years old, 20 females) were fingerprinted with LC-QTOF-MS. Patients also underwent PET/MRI examination. Pearson correlation (absolute $r>0.4$ and $p\leq0.05$ were considered significant) was used to evaluate a relation between intensities of metabolites and MTV, SUV maximum and SUV mean values. Several bile acids (r=-0.42-0.51, p=0.0006-0.002), sulfated steroid hormones (r=-0.41-0.55, p=0.00006-0.005), amino acids (r=-0.42-0.58, p=0.00002-0.003), and acylcarnitines (r=-0.42-0.54, p=0.0001-0.003) were found correlated with tumor size and activity. Differences in correlation of metabolites with MTV and SUV values were observed for uric acid (r=-0.36, p=0.01 vs r=-0.44, p=0.001), oleamide (r=0.42, p=0.03 vs r=0.2, p=0.2), anandamide (r=-0.42, p=0.003 vs r=-0.21, p=0.1), and several lysophosphatidylethanolamines (r=-0.4-0.55, p=0.00006-0.001 vs r=-0.25-0.37, p=0.09-0.01). Metabolic activity of tumor affects bile acids, amino acids, steroid hormones and energy metabolism. Tumor growth mainly affects lysophosphatidylethanolamines metabolism. Acknowledgment: The study was funded by the grant from National Centre for Research and Development in the framework of Programme “Prevention practices and treatment of civilization diseases”–STRATEGMED (STRATEGMED2/266484/2/NCBR/2015).

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BIOMEDICAL

P-41 Small-molecule mutant isocitrate dehydrogenase inhibitors diminish metabolic differences between mIDH1 and wIDH1 expressing cells

PRESENTING AUTHOR: ***Ingvild Comfort Hvinden, University of Oxford, Department of Chemistry, United Kingdom***

CO-AUTHORS: ***John Walsby-Tickle, Christopher J. Schofield, James S. O. McCullagh***

Oncogenic missense mutations in isocitrate dehydrogenase (IDH) 1 or 2 have been found in at least 13 different cancers. These mutations leads to metabolic and epigenetic perturbations, including a substantial increase in the oncometabolite R-2-hydroxyglutarate (2HG), which is considered key to tumorigenesis. In lower grade gliomas, approximately 70% of tumours express mIDH1 or mIDH2 but they remain challenging to diagnose early and treat; mIDH has emerged as a promising target. The goal of this study was to better understand the effect of small-molecule IDH1 inhibitors (AGI-5198, AGI-120, AGI-881) on altered metabolic pathways in IDH mutant and wild type cells. Glioblastoma cells (LN18) were genetically engineered to express IDH1 R132H; cell metabolism was investigated using anion exchange and reversed phase chromatography coupled to mass spectrometry. Treatment of mIDH cells revealed a substantial decrease in 2HG. Additionally, 2-oxoglutarate, β-citryl-glutamate, saccharopine, 2-aminoadiapate, and oxoadipic acid were elevated upon treatment of mutant cells. The latter three metabolites are intermediates in lysine metabolism, a pathway which appears to be significantly affected in mIDH1 cells. B-citryl-glutamate is an endogenous iron chelator and has been associated with regulation of neuronal cell differentiation. These findings suggest that IDH inhibitors are able to 'normalise' altered mIDH1 metabolism, bringing it closer to wIDH1 metabolism. The consequences of such 'normalisation' are yet to be understood from a therapeutic perspective, but the metabolic adaptations in response to treatment could help identify metabolic targets for therapeutic intervention, e.g. the lysine degradation pathway.

P-42 Predictive biomarker discovery of treatment response in lung cancer: A metabolomic approach

PRESENTING AUTHOR: ***José Pérez, MEDINA, Spain***

CO-AUTHORS: ***José Pérez del Palacio, Caridad Díaz-Navarro, Leticia Díaz-Beltrán, Ariadna Martín-Blázquez, Daniel Franco, Ana Laura Ortega-Granados, María Ruiz, Mónica Fernández, Capilla De la Torre, Francisco José García-Verdejo, Francisca Vicente, Olga Genilloud, Pedro Sánchez-Rovira***

Although the broad range of chemotherapeutic agents approved in the late years, it is a challenge for oncologists to choose which drug or combination of drugs will represent the best option for each individual, since only a portion of patients will respond properly. In this regard a biomarker approach to predict patient response to treatment, may be very helpful in the making decision process. Metabolomics, the unbiased identification and quantification of small molecule metabolites in biological samples, is particularly promising for biomarker development because altered metabolism is considered a hallmark of cancer. In this work, we have investigated the metabolome of an initial set of 115 plasma samples from lung cancer patients by mean of liquid chromatography high resolution mass spectrometry. The obtained data matrix was analyzed according the clinical response to each therapy (neoadjuvant and immunotherapy) in order to search for a predictive molecular signature in each group of patients. As a result, multivariate ROC analysis displayed moderate ability to discriminate between Responder and Non-responder lung cancer patients. We found and tentatively identified 1 compound that was differentially regulated in lung cancer patients with complete clinical response vs. progressive disease patients. This study has been currently increased with a second batch of 100 samples of lung cancer patients with the objective of confirm our previous findings. These results suggest that metabolomics might provide a new era of response biomarkers, more appropriate for monitoring therapy.

P-43 Highly untargeted lipidomics of NSCLC shows differentially abundant lipid classes in cancer vs non-cancer tissue

PRESENTING AUTHOR: ***Hunter Moseley, University of Kentucky, United States***

CO-AUTHORS: ***Joshua M. Mitchell, Robert M. Flight***

Lung cancer is the leading cause of cancer death worldwide and non-small cell lung cancer (NSCLC) represents 85% of newly diagnosed lung cancers. The high mortality rate is due in part to the lack of effective treatment options for advanced disease. Incomplete understanding of NSCLC metabolism at a molecular level limits the development of effective treatments. Ultra-high-resolution Fourier transform mass spectrometry (FTMS) combined with our untargeted assignment tool SMIRFE enable a systematic and less biased examination of NSCLC lipid metabolism. From 86 patients with suspected resectable stage I or IIa primary NSCLC, lipid extracts were prepared from paired disease and non-disease tissue samples and analyzed using FTMS. Pathological examination revealed that the majority of the samples were primary NSCLC. Machine learning was then employed to classify SMIRFE formula assignments into lipid categories, followed by differential abundance analysis. Both sterols and glycerolipids were consistently and significantly upchanged in disease versus non-disease. The significant sterol upchange suggests a possible therapeutic role for statins and nitrogenous bisphosphonates, inhibitors of endogenous sterol biosynthesis, in the treatment of primary NSCLC. Additionally, the sterol esters are consistently and significantly upchanged, suggesting increased SCD1 activity. SCD1 expression is a known negative prognostic indicator for survival in NSCLC. In our study, a large fraction of the NSCLC samples displayed this phenotype, suggesting that this metabolic phenotype is shared across multiple genetic subtypes. Thus, inhibitors of SCD1 and other enzymes involved in the production of this metabolic phenotype could have utility in the treatment of many NSCLC genetic subtypes.

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BIOMEDICAL

P-44 What can we learn about differences in metabolic profiles of urinary tract cancers? Multiplatform approach

PRESENTING AUTHOR: ***Marta Kordalewska, Dept. of Biopharmacetics and Pharmacodynamics, Medical University of Gdansk, Poland***
CO-AUTHORS: ***Julia Jacyna, Renata Wawrzyniak, Marcin Markuszewski, Marcin Matuszewski, Michał Jan Markuszewski***

Standard methods utilized in diagnostics of urinary tract cancers require the use of specialist equipment, may cause patients' discomfort and are adopted when disease symptoms are observed, mostly at the late stage of the disease. As Bladder cancer (BCa) and Renal Cell Carcinoma (RCC) constitute ninth and thirteen types of cancer in terms of incidences worldwide, respectively [1], specific and non-invasive diagnostic methods for early diagnosis of BCa and RCC are needed. Urine samples obtained from BCa patients and healthy volunteers were analyzed with the use of LC-MS and GC-MS. The obtained datasets were subjected to deconvolution, filtration and normalization. Afterwards, statistical analysis was utilized to select metabolites that represented significant differences between studied groups. Samples obtained from RCC patients were analyzed in analogical manner. Finally, the identification of selected metabolites was performed with the use of publicly available databases allowing for creation of lists of potential metabolic indicators of BCa and RCC, that were compared for similarities (e.g. altered concentrations of hippuric acid and uridine). The obtained results suggest that urine metabolic fingerprinting could be a powerful tool for metabolic indicators' investigation and searching for explanation of BCa and RCC pathomechanisms at molecular level. Acknowledgements: The project was supported by the National Science Centre grant no. 2015/19/N/NZ7/03397

P-45 Characterization of Plasma-derived Exosomal Proteins Biomarkers in Glioma Patients

PRESENTING AUTHOR: ***Rashmi Rana, Sir Ganga Ram Hospital,Old Rajinder Nagar, India***
CO-AUTHORS: ***Jyoti Kumari***

Exosomes in Glioma are distinctly strong intermediator with ability for flipping the action of neighbouring cells. This becomes more obvious that exosomes have aptitude to encourage the development of pre metastatic niche. As for metastasis to happen, glioma cells need to migrate in a new environment which also requires being favourable for tumor to colonize properly. Their release into the circulation has the potential to inform about tumor status. In-depth proteomic characterization of plasma-derived EVs has been limited by challenges in isolating EVs from protein-abundant biological fluids. We implemented a novel single-step density gradient flotation workflow for efficient and rapid isolation of highly enriched circulating EXO from plasma-derived exosomes. Effective methods for glioma are still in the waiting and there is strong need for high confidence identification of blood-based biomarkers. Exosomes have recently emerged as a novel source of circulatory biomarkers for cancer. We assess the value of exosome proteins as biomarkers for glioma. NTA and TEM confirmed the presence of plasma-derived EXOs isolated by differential centrifugation. Total proteins extracted from plasma-derived EXOs of 40 glioma (grade I, II, III, IV) and 40 healthy subjects was analyzed. FACS analysis to characterized exosomal proteins tetraspanins CD63, CD9 and CD81 was demonstrated to be significantly increased in glioma patients with controls.1D analysis of exosomal proteins was carried out to identify proteins showing altered levels in glioma cases in comparison to controls. Our findings support the potential of exosomes derived proteins as a source of biomarkers that complement other approaches for tumor assessment.

P-46 Sequential untrageted metabolomics and lipidomic profiling identifies key difference in amino acid and lipid between the bone marrow plasma of MGUS and Multiple Myeloma

PRESENTING AUTHOR: ***Wilson Gonsalves, Dr., United States***
CO-AUTHORS: ***Kasia Broniowska, Erik Jessen, Xuan-Mai Petterson, Shaji Kumar, Sreekumaran Nair***

Multiple myeloma (MM) is a clonal plasma cell malignancy that is incurable and has a devastating clinical course. It is always preceded by an asymptomatic precursor disorder, monoclonal gammopathy of undetermined significance (MGUS). We conducted a prospective assessment of metabolome differences between the bone marrow plasma obtained from patients with MGUS to that of patients with MM. Global metabolite profiling was performed by Metabolon Inc. on the bone marrow plasma of 25 MGUS patients and 25 MM patients using a Waters ACQUITY ultra-performance liquid chromatography (UPLC) and a Thermo Scientific Q-Exactive high resolution/accurate mass spectrometer interfaced with a heated electrospray ionization (HESI-II) source and Orbitrap mass analyzer operated at 35,000 mass resolution. Lipidomics was performed on a Shimazdu LC and the Sciex SelexIon-5500 QTRAP. Following log transformation and imputation of missing values, if any, with the minimum observed value for each compound, ANOVA contrasts were used to identify biochemicals that differed significantly between the MGUS and MM groups. Principal component analysis (PCA) and hierarchical clustering analysis (HCA) were able to show group separation based on several distinct classes of metabolites. Overall, it was determined that bone marrow plasma samples obtained from patients diagnosed with MM and MGUS, differed in a number of metabolic readouts, including changes in lipid metabolism, amino acid catabolism, and nucleotide turnover. Verification of these results in an independent cohort is required next to lead to a panel of biomarkers that could aid in identifying possible predictive biomarkers of progression from MGUS to MM.

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BIOMEDICAL

P-47 Serum and tissue metabolomics for assessment of treatment response and survival of breast cancer patients

PRESENTING AUTHOR: ***Julia Debik, Department of Circulation and Medical Imaging - MR Center, Faculty of Medicine and Health Sciences, Norway***

CO-AUTHORS: ***Leslie R. Euceda, Steinar Lundgren, Olav Engebraaten, Øystein Garred, Hedda von der Lippe Gythfeldt, Tone F. Bathen, Guro F. Giskeødegård***

While tissue metabolomics reveals the direct effect of treatment, serum metabolomics shows a snapshot of the metabolic state of the host organism, affected by factors such as diet, age and treatment, in addition to the tumor itself. The aim of this study is to characterize the metabolic profile in breast cancer patients receiving neoadjuvant chemotherapy (N=132) and to relate metabolic changes to treatment response and survival. Half of the patients were randomized to receive bevacizumab (antiangiogenic drug) in addition to standard chemotherapy, and tumor biopsies and serum were collected prior to and during treatment. All samples were analyzed by nuclear magnetic resonance spectroscopy (NMR). Multilevel partial least squares discriminant analysis (multilevel PLS-DA) showed clear changes in serum metabolite levels during treatment (p-values ≤ 0.01). Increased levels of isoleucine and decreased levels of creatine, creatinine, histidine and ornithine, in addition to unfavorable changes in lipid levels were observed between pre- and on-treatment samples. Bevacizumab treatment and poor response (patients with residual cancer burden (RCB) index 3) were associated with the serum metabolic profile (accuracies = 64%, 64%; p-values = 0.014, 0.049, respectively). Five-year survival could be predicted from metabolic profiles in tissue (accuracy = 72%; p-value = 0.005), but not serum. Low correlations between serum and tissue metabolites were observed, revealing the complementary nature of the metabolic information in these biological matrices. Collectively, our results demonstrate a potential clinical application of serum and tissue metabolomics for patient-monitoring during and after treatment, further indicating potential for NMR in assessment of patient outcome.

P-48 A Coupled Lipidomics-Machine Learning Approach for Early Diagnosis of clear cell Renal Cell Carcinoma

PRESENTING AUTHOR: ***María Monge, CIBION-CONICET, Argentina***

CO-AUTHORS: ***Malena Manzi, Martín Palazzo, Nicolás Zabalegui, María Elena Knott, Pierre Beauseroy, Patricio Yankilevich, María Isabel Giménez***

Clear cell (ccRCC) is the most common (75%) lethal subtype of RCC, and is considered a glycolytic and lipogenic tumor. More than 30% of patients, often incidentally diagnosed by imaging procedures, exhibit locally advanced or metastatic RCC at the time of diagnosis and the disease is inherently resistant to chemotherapy and radiotherapy. In this work, serum samples from a cohort that included patients with different ccRCC stages (SI, SII, SIII and, SIV; n=112) and controls (n=52) were interrogated with a discovery-based lipidomics approach using ultraperformance liquid chromatography coupled to quadrupole-time-of-flight mass spectrometry and machine learning methods. Support vector machine models and the least absolute shrinkage and selection operator (Lasso) variable selection method yielded two discriminant panels for ccRCC detection and early diagnosis. A first 18-feature panel allowed discriminating ccRCC patients from controls with 96.8% accuracy in a training set under cross-validation, and 81.4% accuracy in an independent test set. Fifteen features of the panel were significantly decreased in ccRCC after correcting for multiple testing. A second model was trained to discriminate early stages (I-II) from late stages (III-IV) ccRCC and provided a 26-feature panel that allowed sample classification with 84.5% accuracy in the training set under cross-validation, and 82.1% accuracy in the classification of stage I ccRCC patients from an independent test set. Current work involves feature identification in both panels by accurate mass, isotopic pattern, tandem MS experiments, and chemical standard analysis. Results are auspicious for early ccRCC diagnosis after validation of the panels in larger and different cohorts.

P-49 Untargeted metabolomics study of urine and plasma samples in prostate cancer patients

PRESENTING AUTHOR: ***Wiktoria Struck-Lewicka, Medical University of Gdansk, Poland***

CO-AUTHORS: ***Małgorzata Patejko, Renata Wawrzyniak, Marta Kordalewska, Danuta Siluk, Marcin Markuszewski, Marcin Matuszewski, Michał Markuszewski***

Prostate Cancer (CaP) is the second most common type of cancer and the third leading cause of cancer death in men. Despite its common occurrence and high mortality rate, the currently used CaP biomarkers are still not enough specific and selective. Therefore, there is an urgent need for searching cancer related metabolites that would support in explaining pathogenesis of the disease and can be considered as potential markers in CaP recognition. In this project, we performed analyses of metabolic fingerprints from plasma and urine samples derived from prostate cancer patients (n= 40) and healthy volunteers (n=43). The determinations were utilized using complementary analytical techniques, namely gas chromatography (GC-EI-QqQ/MS) and liquid chromatography (LC-ESI-TOF/MS) both coupled with mass spectrometry detection. Each data set was pre-processed, filtered and aligned. Next, the univariate and multivariate statistical analyses were carried out for selection of statistically significant metabolites (FDR p value <0.05) and subsequently in order to assess their prediction ability. Metabolites were putatively identified using several databases including HMDB, KEGG, Metlin and LipidMaps. The selected significantly altered metabolites that contribute the most into groups discrimination were mainly linked to amino acid, TCA cycle, FFA and purine and pyrimidine metabolism [1]. These results have to be further confirmed using targeted metabolomics approach. The work has been supported by the National Science Centre by the project no 2014/13/D/NZ7/00368.

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BIOMEDICAL

P-50 Unravelling altered RNA metabolism in pancreatic cancer and breast cancer cells by advanced mass spectrometric techniques

PRESENTING AUTHOR: *Simon Lagies, Center for Biological Systems Analysis, University of Freiburg, Germany*

CO-AUTHORS: *Manuel Schlimpert, Lukas M. Braun, Michel Kather, Michael Rodamer, Thalia Erbes, Uwe A. Wittel, Bernd Kammerer*

Modified nucleosides are a diverse class of chemically similar compounds where the common nucleosides bare additional functional groups such as methylations, acetylations or hydroxylations. They are produced under physiological conditions but are especially elevated in transformed cells or tissues. They cannot be recycled by the cellular salvage pathway and are therefore excreted to cell culture medium or body fluids. Here, we compare the profiles of excreted modified nucleosides in different pancreatic as well as breast cancer cell lines obtained by LC-QqQ or LC-IMS-HRMS analysis. Cluster analyses are capable of discriminating even different sub-types of these malignancies. Thus, monitoring modified nucleosides is of high potential in future cancer research.

P-51 Phenotyping of cellular senescence by 1H-NMR based metabolomics and hyperpolarized 13C-MRS

PRESENTING AUTHOR: *Christoph Trautwein, University of Tuebingen, Werner Siemens Imaging Center, Germany*

CO-AUTHORS: *Marie-Aline Neveu, Ben Zhou, Marcel A. Krueger, Lars Zender, Andreas M. Schmid, Bernd J. Pichler*

Senescence is a steady state of cell cycle arrest and an often observed phenomenon in cancer. While it can be seen as a stable end-point for the disease, still little is known about detailed mechanisms during therapy. The investigation of senescence metabolism may therefore help to develop new in-vivo markers and treatment approaches. In this study we investigated metabolic changes in different senescent cancer cell models using in-vitro hyperpolarized 1-13C-pyruvate MRS (7T) and ex-vivo 1H-NMR (600 MHz). Senescence was induced in human colon cancer cells (HCT116) by doxorubicin, in liver progenitor cells (HRas) by p53-reactivation and in a liver carcinoma cell line (HCC) by a ribosomal checkpoint inhibitor (RCI). Experiments with hyperpolarized 1-13C-pyruvate revealed a significantly decreased lactate production in RCI treated HCC cells without changes in alanine production. Interestingly, lactate and alanine metabolism were not impaired in senescent HCT116 cells. These findings are supported by results from 1H NMR data for metabolites of cell growth and energy storage. A 2-fold lactate/alanine increase and 3-fold phosphocholine/glycerophosphocholine decrease was identified for HRas compared to HCT116 cells between control and senescence. By contrast, ratios of energy storage (phosphocreatine/creatine) were similar between the two cell lines and applied therapy. Our first data suggest that senescence metabolism is influenced by treatment type as well as genotype. RCI inhibition and p53-reactivation impaired lactate production in HCC and HRas progenitor cells, respectively, while doxorubicin didn't in HCT116 cells where cell growth markers remained high. Further investigations of lipid metabolism and additional sampling time points are required.

P-52 One carbon metabolomics in cancer

PRESENTING AUTHOR: *Matthias Schittmayer, OMICs Center Graz, Austria*

CO-AUTHORS: *Martin Pichler, Joerg Lindenmann, Luka Brcic, Nicola Zamboni, Ruth Birner-Gruenberger*

One carbon metabolism is a limiting pathway in cellular proliferation as it is crucial for the biosynthesis of nucleotides. Therefore, one carbon metabolism has continuously been a major target of cancer chemotherapy from its beginning in the 1950s until the present day. Also, altered expression levels of individual one carbon metabolism enzymes have been reported as highly significant biomarkers of drug efficacy and disease free survival in various types of cancer. However, enzyme abundance is only an indirect readout of enzymatic activity and the resulting metabolome. So far, metabolic analysis of one carbon metabolism has been hampered by the low chemical stability and high interconvertibility of folate cofactors, which play a crucial role in the transfer of one carbon units. We have developed a complete workflow that employs simultaneous extraction and stabilization of folates by derivatization that allows a comprehensive assessment of metabolites in one carbon metabolism and the connected pathways. We now aim to apply this method on non-small cell lung carcinoma to classify individual tumors based on one carbon metabolism patterns and predict chemoresistance/sensitivity as well as tumor differentiation grade and invasiveness, to enable precision treatment.

P-53 Reactivation of Dihydroorotate Dehydrogenase-Driven Pyrimidine Biosynthesis Restores Tumor Growth of Respiration-Deficient Cancer Cells

PRESENTING AUTHOR: *Sehyun Oh, Seoul National University, South Korea*

CO-AUTHORS: *Martina Bajzikova, Jaromira Kovarova, Ana R. Coelho, Stepana Boukalova*

Cancer cells without mitochondrial DNA (mtDNA) do not form tumors unless they reconstitute oxidative phosphorylation (OXPHOS) by mitochondria acquired from host stroma. To understand why functional respiration is crucial for tumorigenesis, we used time-resolved analysis of tumor formation by mtDNA-depleted cells and genetic manipulations of OXPHOS. We show that pyrimidine biosynthesis dependent on respiration-linked dihydroorotate dehydrogenase (DHODH) is required to overcome cell-cycle arrest, while mitochondrial ATP generation is dispensable for tumorigenesis. Latent DHODH in mtDNA-deficient cells is fully activated with restoration of complex III/IV activity and coenzyme Q redox-cycling after mitochondrial transfer, or by introduction of an alternative oxidase. Further, deletion of DHODH interferes with tumor formation in cells with fully functional OXPHOS, while disruption of mitochondrial ATP synthase has little effect. Our results show that DHODH-driven pyrimidine biosynthesis is an essential pathway linking respiration to tumorigenesis, pointing to inhibitors of DHODH as potential anti-cancer agents.

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*AWARD WINNERS

BIOMEDICAL

P-54 In-vitro anticancer activity of Flower of Cassia alata L. on Human urinary bladder carcinoma (T24) cell line

PRESENTING AUTHOR: ***Meenakshi Barua, B.K. Birla College of Arts, Science and Commerce., India***

CO-AUTHORS: ***Meeta Bhot***

The present study investigated Cassia alata L. Flower plant fraction on human urinary bladder carcinoma (T24) cell lines via in-vitro SRB assay for anticancer property. Fresh flowers of Cassia alata (C. alata) plant were collected from suburban area of Maharashtra, India for preparation of plant extract via Soxlet extraction method. Bioactive fraction was separated via Column Chromatography method. Separated fraction was used against T24 carcinoma & Vero cell line (mammalian non cancerous cells) in four different concentration viz. 10, 20, 40, 80µgml. Effect was compared with Adriamycin (standard drug, used as positive control) and Emodin, a Plant standard. Separated plant fraction demonstrated a dose-dependent reduction in the overall activity of T-24 carcinoma cell line. C. alata flower separated fraction exhibited cytotoxic effect on T24 carcinoma cell line with IC50 value 45.3µg/ml and effective GI50 value 18.8µg/ml. Graph obtained from SRB cytotoxicity assay showed that separated plant fraction reduced the growth in T24 cell lines by lower than 50% with initial 10µg/ml concentration. In Vero cell line separated plant fraction and plant standard not showed any cytotoxic effect even when applied in higher concentration i.e. 80µg/ml. In contrast, standard anticancer drug showed pronounced cytotoxic effect even in lower concentration i.e. 10µg/ml. Phase contrast micrographs of T24 cell line treated with separated fraction showed significant decrease in viable cell numbers at 24 hrs of treatment. In conclusion, present study showed that separated fraction from C. alata flower has potential anticancer activity and showed significant result against Urinary bladder carcinoma cell line.

P-55 Colorectal Cancer : Biomarkers and Effect Size

PRESENTING AUTHOR: ***Nicolas Di Giovanni, University of Liège, MS Lab, Obiachem Group, Belgium***

CO-AUTHORS: ***Meuwis Marie-Alice, Louis Edouard, Focant Jean-François***

Colorectal cancer kills more than 700.000 persons each year worldwide. Nevertheless, its diagnosis is still largely based on invasive tissue sampling and gaps remain in the understanding of its pathogenesis, with complex combinations between lifestyle, genetics, epigenetics, chronic inflammation (IBD) and microbiota. We analyzed serum samples from patients affected by colorectal cancer (CRC, n = 18) and by colorectal cancer in remission (R-CRC, n = 17), and samples from healthy patients matched for biases (HC, n = 19 and R-HC, n = 17). The aim was to find candidate biomarkers able to diagnose the active state of the disease as well as to compare the concentration levels of the molecules of interest with the remission state to better understand the biolocal processes beneath the observed clinical and metabolic symptoms. To do so, an optimized and validated (NIST SRM 1950) comprehensive GC×GC-(HR)TOFMS method we developed was used. It includes an in-house QC system, data processing based on multiple statistical techniques and identification using full mass spectrum, linear retention indices and accurate mass provided by state-of-the-art high-resolution (HR) time-of-flight mass spectrometry. Because the experimental design prevented a direct comparison between the active and remission samples, which were not directly matched for biases, we used a measure called effect size that has the advantage to not only focus on statistical significance but on effect (here signal variation) magnitude. We will discuss the interest and application of effect size in metabolomics and we will present the highlighted candidate biomarkers in terms of discrimination potential.

P-56 An untargeted lipidomic analysis reveals ganglioside GM2/GM3 balance and the regulation of GM2-AP cofactor through GRP94 chaperone as important new key players in brain metastasis

PRESENTING AUTHOR: ***Carmen Bedia, Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Spain***

CO-AUTHORS: ***Míriam Badia, Laia Muixí, Thierry Levade, Romà Tauler, Angels Sierra***

GRP94 is an ATP-dependent chaperone able to regulate pro-oncogenic signaling pathways. Previous studies have shown a critical role of GRP94 in brain metastasis pathogenesis and progression. In this work, we explored if GRP94 regulates lipid metabolism in GRP94-deficient cell models through LC-MS and an untargeted lipidomic analysis using the ROIMCR methodology. The results showed that some lipid species were altered in GRP94-deficient cells, specially GM2 and GM3 gangliosides. GRP94-silenced cells contain lower levels of GM3 and higher levels of GM2 compared to control cells, indicating that the catalytic pathway of GM2 was affected by the low enzymatic activity of β-Hexosaminidase (HexA), responsible for the hydrolysis of GM2 to GM3. Moreover, a deficiency of the GM2-activator protein (GM2-AP), the cofactor of HexA, is observed without any gene expression change, indicating a post-transcriptional alteration of GM2-AP in the GRP94-ablated cells. These results suggest that GM2-AP may be a client of GRP94 chaperone, resulting in defective GM2 catabolic processing and lysosomal accumulation of GM2 in GRP94-ablated cells. Overall, given the role of gangliosides in cell surface dynamics and signaling, their imbalance might be linked to modifications of cell behaviour acquired in brain metastasis progression. On the other hand, this work indicates that GM2-AP is a key factor in the maintenance of ganglioside balance in cell membranes, which is regulated by GRP94 expression. These findings highlight the relevance of GM3 and GM2 gangliosides in BrM and reveal GM2-AP as a promising diagnosis and therapeutic target in BrM research.

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*AWARD WINNERS

BIOMEDICAL

P-57 HR-MAS NMR based metabolic profiling in thymus cancer

PRESENTING AUTHOR: ***Mikheil Gogiashvili, Leibniz Institut für Analytische Wissenschaften - ISAS - e.V., Germany***

CO-AUTHORS: ***Mohammad Al Wahsh, Jörg Lambert, Roland Hergenröder, Djeda Belharrazem, Alexander Marx***

The thymus is an important lymphoid organ that is directly linked to the immune system. Thymus cancer, classified either as thymoma or thymic carcinoma, shows an increasing incidence with age and is so far lacking any curative options except surgery. It is often accompanied by autoimmune diseases, anemia or a lack of antibodies. The molecular pathogenesis of thymic epithelial tumors is hardly understood especially with respect to metastasis formation and tumor recurrence. A more thorough understanding of the molecular processes in thymic tumor tissue would improve the prognosis and would offer new treatment options for thymus cancer. High resolution (HR) magic angle spinning (MAS) nuclear magnetic resonance (NMR) based metabolic profiling is increasingly being used in cancer tissue analysis, for instance to assess the correlation of the metabolic profile with prognostic factors and with clinically relevant endpoints such as survival outcome and therapy response. Since HR MAS measurements are non-destructive, metabolomics studies can be easily combined with further investigations like subsequent histological or other “omics” related analysis, for example to discover molecular subgroups of diseases by combining quantitative metabolic and transcriptomic data. Thymomas and thymic carcinomas are rare tumors (occurrence approx. 1-2 in 500 000). In this study, we for the first time performed non-targeted metabolic profiling on a very valuable cohort of 21 tissue samples of thymus tissue as well as benign and malignant thymomas by means of HR-MAS NMR. The metabolite levels are correlated with clinicopathological parameters like gender, age, histology and Masaoka classification.

P-58 Comparison of plasma metabolite profiles between healthy cohort and cancer patients in Japanese individuals

PRESENTING AUTHOR: ***Eiji Hishinuma, Advanced Research Center for Innovations in Next-Generation Medicine, Japan***

CO-AUTHORS: ***Naomi Matsukawa, Daisuke Saigusa, Bin Li, Keigo Komine, Hidekazu Shirota, Muneaki Shimada, Seizo Koshiba, Masayuki Yamamoto***

Altered cell metabolism, a characteristic feature for many kinds of cancers, results in changes to intracellular and extracellular metabolite concentrations. Although many kinds of metabolites were reported as biomarkers, cancer-related change of metabolism is still not well understood. In order to diagnose and treat cancer patients comprehensively and effectively, it is important to identify specific biomarkers by reliable quantification of metabolites. Our aim is to identify the changes of metabolic profiles in plasma of individuals with cancer in comparison with healthy controls by quantification of many kinds of metabolites. As a first step, plasma concentrations of metabolites including amino acids, biogenic amines, acylcarnitines, glycerophospholipids, sphingolipids, and hexoses were quantified using LC-MS/MS and FIA-MS/MS (AbsoluteIDQ p180 kit). We compared metabolic profiles of plasma samples from cancer patients (gastric, ovarian, colorectal, pancreatic cancer, etc.) with those from healthy controls. As a result of principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA), cancer patients showed different metabolic profiles compared to healthy controls. Plasma concentrations of some amino acids, biogenic amines, and glycerophospholipids were significantly changed in the case of cancer plasma. Especially, tryptophan, histidine, and lysophosphatidylcholines were decreased, while glutamine was increased. These metabolites may be involved in the progression of cancer. Our data suggest that exhaustive profiling of plasma metabolites contributes to personalized medicine for cancer.

P-59 Spatial Information of Metabolites Using Mass Spectrometry Imaging on Breast Needle Biopsy

PRESENTING AUTHOR: ***Vincen Wu, Imperial College London, United Kingdom***

CO-AUTHORS: ***Paolo Inglese, Hui-Yu Ho, Andreas Dannhorn, Emine Kazanc, Goncalo Correia, James McKenzie, Stephanie Ling, Evdoxia Karali, Nikolaos Koundouros, Hiromi Kudo, Peter Kreuzaler, Sami Shousha, Ian Gilmore, Mariia Yuneva, Richard Goodwin, Josephine Bunch, George Poulogiannis, Zoltan Takats***

Breast cancer is one of the most prevalent forms of cancer in women worldwide, and it is a highly heterogeneous and complex disease with distinct molecular features. The gold standard for clinical diagnosis of breast cancer is through histopathology and the presence or absence of certain marker proteins. These methods do, however, not fully capture the biological heterogeneity of the disease. Mass spectrometry imaging is a powerful tool that allows us to produce metabolic images of clinical tissues with their corresponding distribution and intensity. For this study, 12 breast needle biopsies were analyzed and compared using multivariate analysis, towards the profiling of altered metabolites and lipids between different tissue types, to understand the heterogeneity of breast cancer.

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BIOMEDICAL

P-60 Multiplatform metabolomics for the biomarker discovery in pancreatic cancer

PRESENTING AUTHOR: ***Raquel Cumeras, Universitat Rovira i Virgili (URV), Spain***

CO-AUTHORS: ***Raquel Cumeras, Esther Molina-Montes, Alfredo Carrato, Xavier Molero, Francisco X. Real, Núria Malats, Xavier Correig***

Pancreatic ductal adenocarcinoma (PDAC) or pancreatic cancer, is the deadliest cancer worldwide, despite its relatively low population incidence, and early-stages biomarkers are needed. Samples were selected from a large case-control study (4000-subjects) on pancreatic cancer (PanGenEU) conducted in 28-centres from 6-European countries. In this study, metabolomics profiles of 60 PDAC cases and 60 controls from 2-centres in Spain, were evaluated using non-targeted metabolomics with UHPLC-QTOF-MS, GC-QTOF-MS for serum samples (polar and non-polar phases), and 1H-NMR for urine and fecal samples. LC-MS peak peaking, alignment and retention drift correction was done with XCMS, then QC-normalized with PQN. Samples from both centers were analyzed together, randomizing their order in the experimental analysis, so the relevant metabolites identified (FDR<0.05) were not influenced by an experimental batch. The univariate t-test returned 484 relevant features. The features whose m/z returned a hit either in METLIN or HMDB were selected for MS/MS. For GC-MS, data was deconvoluted and aligned with eRah package in R and metabolites were ID with Golm Database. In NMR, to account for the potential dilution effect of urine, metabolites were PQN-normalized and used the kNN-imputation (k=5). Within the multiplatform analysis, the following metabolites were found up-regulated in PDAC samples: 1-methylnicotinamide; various secondary bile acids (such as glycocholic acid, and taurocholic acid); and some sugars (like galactose). With this multiplatform and multifluid results, and relevant clinical and biochemical metadata we will generate a predictive model for PDAC. Model results combining the serum, urine and fecal relevant features would be presented at the conference.

P-61 Metabolome analysis of cancer-derived extracellular vesicles under different oxygen concentration

PRESENTING AUTHOR: ***Akiyoshi Hirayama, Keio University, Japan***

CO-AUTHORS: ***Ryosuke Hayasaka, Masaru Tomita, Tomoyoshi Soga***

Extracellular Vesicles (EVs) are facilitators of cell-to-cell communication. Cancer-derived EVs contribute to cancer progressions such as distant metastasis, angiogenesis, and immunosuppression. EVs contain functional cellular components including DNA, mRNA, microRNA, and protein. However, metabolomic profiling in cancer-derived EVs remains largely unexplored. The purpose of this study is to explain comprehensive metabolomic profiling of pancreatic cancer-derived EVs. As a model for studying cancer metabolism, we evaluate the difference between metabolomic profiles in EVs obtained from cancer cells cultured in normoxic or hypoxic conditions. Pancreatic cancer cell line Panc1 was cultivated under normoxic (20% oxygen) and hypoxic (1% oxygen) conditions. Cells were sampled using methanol and EVs were isolated from conditioned medium using ultracentrifugation. The amount of EVs was determined by nanoparticle tracking analysis and the protein level of the CD9 exosomal marker was measured using enzyme-linked immunosorbent assay (ELISA). Metabolome analysis was performed by using capillary ion chromatography-mass spectrometry and liquid chromatography-mass spectrometry. We identified more than 180 kinds of metabolites in pancreatic cancer-derived EVs. In addition, principal component analysis (PCA) of metabolites in EVs showed some separation between normoxia and hypoxia. These results suggest that the metabolomic profiling of cancer-derived EVs differ different oxygen concentrations.

P-62 The metabolic footprint of intensive weight management in an open-label, cluster-randomised trial (DiRECT)

PRESENTING AUTHOR: ***Caroline Bull, University of Bristol, United Kingdom***

CO-AUTHORS: ***Laura J. Corbin, Michael E. J. Lean, Roy Taylor, Naveed Sattar, Nicholas J Timpson***

Type 2 diabetes is strongly related to weight gain and accumulation of excess fat within the liver and pancreas. Results from the Diabetes Remission Clinical Trial (DiRECT) have shown that a professionally supported intensive weight management programme can enable patients to undergo diabetes remission. In this trial of 298 patients, 46% of those in the intervention arm (68/149) achieved remission at 12 months. Whilst the measurement of standard clinical indicators suggests improved metabolic health in the intervention group, in this work we present a full evaluation of the metabolic consequences of the intervention. Serum samples collected from patients at baseline and one-year have undergone analysis by mass spectrometry (UPLC-MS/MS) and nuclear magnetic resonance (NMR) spectroscopy. In a preliminary analysis of 273 samples, we used a mixed linear modelling approach to compare the levels of 933 MS-derived metabolites in the intervention group (n=125) versus the control group (n=148) at one-year post-intervention. We have shown >350 metabolites to be associated with the intervention including branched chain amino acids (e.g. alpha-hydroxyisovalerate), carbohydrates (e.g. mannose) and lipids, such as sphingomyelins. Integration of our results into a metabolite-disease interaction network indicate potential relevance for cancer and neurological phenotypes. Whilst many of the changes we see are likely due to the intervention effect on weight which was estimated to be -8.8kg (95% CI: -10.3, -7.3), there is also some indication that the metabolite profile reflects dietary and lifestyle changes. These experimental study data on diabetes remission augment the understanding gained from observational studies of diabetes development.

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***AWARD WINNERS**

BIOMEDICAL

P-63 Targeted Multi-OMICS: Rapid plasma profiling of a bladder and lung cancer human cohort

PRESENTING AUTHOR: ***Billy Molloy, Waters Corporation, United Kingdom***

CO-AUTHORS: ***Sarah Lennon, Lee Gethings, Robert Plumb***

Cancer is a complex, life threatening disease, existing in many forms. Here, we present a study comparing plasma samples from cohorts of bladder and lung cancer patients, with healthy controls using a high-throughput targeted OMICS workflow. This workflow allows for rapid screening of various compound classes using a single LC-MS platform. A known level of a labelled analogue from each compound class was added to each sample. The level of each analyte was then estimated using its ratio to this. Data was collected for 18 samples (6 controls; 6 bladder cancer; 6 lung cancers). QC's (a pool from all samples) were acquired every ninth injection. In total, 206 injections were performed (runtime = 22hr). 128 compounds were measured, generating a %CV <20% for the QC samples: 80 proteins, 20 acylcarnitines and 28 amino acids. Valine-d8 (amino acid spiked in samples) is used as an example to illustrate the consistency of response across the whole study (CV= <5%). Pair-wise comparisons using a t-test were performed on each compound class. The amino acid sarcosine was demonstrated to be up-regulated in both lung and bladder cancer samples; the acyl carnitine Octenoyl-carnitine (C8:1) was down-regulated in bladder cancer subjects; and protein analysis highlighted various differentiating species, including Apolipoproteins. This data demonstrates the ability of this targeted approach to highlight potential markers in a high-throughput manner. Method development was unnecessary, and results were obtained in <24 hours. The next step would be to validate the markers by performing a more statistically rigorous study.

P-64 Rational Metabolic Engineering – Metabolism Driven Strategy for Optimization of Cancer Treatment.

PRESENTING AUTHOR: ***Anna Halama, WCMQ, Qatar***

CO-AUTHORS: ***Shaima Dib, Iman Achkar, Sara Kader, Michal Kulinski, Gabi Kastenmueller, Olivier Elemento, Steven S. Gross, and Karsten Suhre.***

The metabolic processes of a cancer cell in comparison with a normal cell are strongly deregulated, and could be associated with increased energetic and adaptive needs required for proliferation, progression and metastasis in challenging microenvironment. Therefore, altered cancer metabolism become an attractive target for therapeutic intervention. For instance, glutaminase inhibitors, same as fatty acid synthesis inhibitors, have shown efficacy in preclinical testing and entered already clinical trials. However, given the complexity of metabolic interactions it could be hypothesized that inhibition of one metabolic pathway might trigger series of metabolic adaptations towards cancer cell survival and as a result end up with a treatment failure. Here, we are proposing to monitor metabolic landscape of cancer cell at its baseline as well as under treatment to provide further insight into cancer metabolic plasticity, which might lead to prediction of rational intervention in the cancer metabolism and thus treatment optimization. We have determined metabolic baseline of 6 normal and 30 cancer cell lines from lung and breast using non-targeted metabolic profiling. We identified 380 metabolites out of which 130 molecules, from various metabolic classes, showed significant deregulations across the cancer cell lines in comparison to normal cells. We further draw a map of cancer cell metabolism and determined key metabolic deregulations. Here, we report on metabolic plasticity under glutaminolysis inhibition and characterize strategies for treatment optimization driven by metabolic analysis. With this study we are proposing approach for optimization of cancer treatment strategies based on metabolic responses, which we call Rational Metabolic Engineering.

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***AWARD WINNERS**

BIOMEDICAL

P-65 Quantitative analysis of 2-hydroxyglutarate in malignant gliomas

PRESENTING AUTHOR: ***Peter Bystrický, Jessenius Faculty of Medicine in Martin Comenius University, Slovakia(Slovak Republic)***

CO-AUTHORS: ***Simona Holubčíková, Ivana Kašubová, Romana Richterová, Branislav Kolarovszki, Zora Lasabová, Peter Račay***

Concentrations of D- and L- 2-hydroxyglutarate (2-HG) were determined in tumor tissue samples of glioma patients WHO grade I – III (astrocytoma, oligodendroglioma, secondary glioblastoma) with possibility of genetic mutation on isocitrate dehydrogenase (IDH) 1 or 2 in vitro. The concentrations were determined (quantified) by HPLC-HRMS technique using Agilent HPLC and Bruker Q-TOF HRMS spectrometer setup. The D-2-HG concentration was double checked by D-2-Hydroxyglutarate Colorimetric Assay Kit. Concurrently the genetic mutation analyses of both IDH 1 and 2 were performed by isolation of cancer tissue DNA, PCR amplification and subsequent Sanger sequencing. This was to confirm the correlation of 2-HG in vitro concentrations in tumor tissue and the results from mutation analyses. Also the in vitro HPLC-HRMS data were correlated with immunohistological findings.

P-66 Metabolomic profiles throughout the continuum of colorectal carcinogenesis: a targeted metabolomics approach

PRESENTING AUTHOR: ***Stefanie Brezina, Institute of Cancer Research, Medical University of Vienna, Austria***

CO-AUTHORS: ***Stefanie Brezina, Andreas Baierl, Anne J.M.R. Geijsen, Eline van Roekel, Pekka Keski-Rahkonen, David Achaintre, Martijn Bours, Fränzel J.B. van Duijnhoven, Biljana Gigic, Tanja Gumpenberger, Andreana N. Holowatyj, Dieuwertje E.G. Kok, Annaleen Koole, Arve Ulvik, Nivonirina Robinot, Jennifer Ose, Alexis B. Ulrich, Per Magne Ueland, Ellen Kampman, Matty Weijenberg, Nina Habermann, Augustin Scalbert Cornelia M. Ulrich, Andrea Gsur***

BACKGROUND. Colorectal cancer is a major public health concern worldwide, remaining the third leading cause of cancer-associated mortality. Colorectal cancer has a natural history of evolution from normal mucosa to adenoma to cancer, thereby providing a window of opportunity for effective intervention and prevention. Thus, the discovery of biomarkers that aid in the characterization of risk of progression along the colorectal carcinogenesis are urgently needed to tailor prevention strategies. METHODS. Targeted metabolomics analysis was performed on plasma samples from 831 newly diagnosed colorectal cancer patients, 600 adenoma patients, and 753 controls, using mass spectrometry and the Biocrates AbsoluteIDQ p180 Kit. Samples included in this study were derived from five colorectal cancer cohorts embedded in the European MetaboCCC consortium. We applied a comprehensive data analysis strategy to investigate signals of changes in the metabolomic profile that vary along the continuum of colorectal carcinogenesis. RESULTS. Data on 131 metabolites were applicable for further analysis in all study samples. We established a categorical regression model to identify metabolites associated with the development of colorectal cancer. Metabolites significantly associated with colorectal carcinogenesis were further used to establish a random effects model able to predict colorectal cancer patients, adenoma cases and controls. CONCLUSION. The complementary study population, brought together as part of the MetaboCCC consortium, provided a unique resource for comprehensive investigations enabling a distinction of malignancy potential. Our findings suggest that metabolites associated with the risk for colorectal carcinogenesis can be used in future for risk stratification, e.g., for tailored screening strategies by endoscopy.

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*AWARD WINNERS

BIOMEDICAL

P-67 Spatial differentiation of metabolism in prostate cancer tissue detected with mass spectrometry imaging

PRESENTING AUTHOR: ***Maria K. Andersen, Department of Circulation and Medical Imaging, Norwegian University of Science and Technology, Norway***

CO-AUTHORS: ***Therese Stork Høiem, Benjamin Balluff, Marta Martin-Lorenzo, Elin Richardsen, Britt Claes, Helena Bertilsson, Ron Heeren, Morten B. Rye, Guro F. Giskeødegård, Tone F. Bathen, May-Britt Tessem***

Molecular profiling of prostate cancer tissue is challenging due to the heterogeneous tissue composition. Mass spectrometry imaging (MSI) enables identification of spatial distribution of biological molecules on tissue sections. In this study, we performed metabolic profiling of 45 prostate cancer tissue samples using matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) MSI (negative and positive ion mode, 30 µm resolution). By pairwise comparing spectra from different tissue types (benign epithelium (BE), cancer epithelium and stroma) with multivariate orthogonlized partial least squares discrimination analysis (OPLS-DA) modeling, we identified several differences in metabolism. The antioxidant-related metabolites taurine and oxidized glutathione had higher levels in stroma compared to benign epithelium. Citrate and spermine had reduced levels in stroma and in cancer epithelium compared to BE, which confirms previous findings. Lastly, carnitine and acetylcarnitine, which are parts of the carnitine shuttle, had higher levels in cancer epithelium compared to BE, while no differences were observed between stroma and BE. The carnitine shuttle is important for the regulation of β-oxidation of lipids vs. lipid synthesis balance. Mass identity of the metabolites was verified through MS/MS. In conclusion, we identified alterations in key molecular processes, such as lipid β-oxidation, prostatic secretory function and inflammation, in prostate tumor tissue using MALDI-TOF MSI. The significant differences in metabolite distributions between the defined tissue structures, pinpoint the importance of access to methodology capable of providing spatial information.

P-68 Comparison of tissue sample preparation methods for comprehensive LC-MS metabolomic analysis of gastrointestinal stromal tumour

PRESENTING AUTHOR: ***Szymon Macioszek, Medical University of Gdansk, Poland***

CO-AUTHORS: ***Urszula Kijanko, Michał Jan Markuszewski***

Gastrointestinal stromal tumour (GIST) is a rare type of cancer that affects around 15 people per million in Europe. Most often GIST develops as a consequence of mutations in KIT gene or PDGFRA gene, less frequently. However, metabolic patterns during GIST development have not yet been investigated and elucidated. To acquire knowledge about changes in biochemical pathways in the cancer tissue, we chose to apply untargeted metabolomics with the use of LC-MS. The first stage of the study was focused on selecting the most appropriate sample preparation method. Due to limited availability of GIST tissue samples, we aimed at developing a method that provides possibility of extracting both polar and lipophilic metabolites from one small GIST specimen. Tissue samples were homogenized with a 50:50 mixture (v/v) of methanol and water in ratio 1:10 (w/v) and obtained homogenate was subjected to five different methods of extraction. They included monophasic extraction with methyl tert-butyl ether (MTBE) and methanol, biphasic extraction with different ratios of MTBE, methanol and water as well as with dichloromethane, methanol and water, and two-step extraction with the same solvents. Organic extracts were analyzed with reversed phase LC-TOF-MS, while HILIC separation was used for polar extracts. Criteria for sample preparation method selection were based on the number of features detected in the extracts and reproducibility of extraction procedure. The selected method will be further used in metabolomic fingerprinting of GIST tissue specimens, which can complement genomic studies on GIST or propose potential new therapeutic targets.

P-69 Method development for determination of modified nucleosides and deoxynucleosides in urine and plasma samples

PRESENTING AUTHOR: ***Malgorzata Patejko, Medical University of Gdańsk, Poland***

CO-AUTHORS: ***Wiktoria Struck-Lewicka, Danuta Siluk, Marcin Markuszewski,***

Modified nucleosides and deoxynucleosides are products of RNA and DNA turnover. They are not metabolized and cannot be utilized for synthesis of RNA and DNA molecules. They are excreted in unchanged form. Consequently, a correlation between their elevated level in urine and pathophysiological disorders development can be expected. Increased level of modified nucleosides and deoxynucleosides was observed in such diseases as: hepatocellular carcinoma, breast cancer or urogenital cancer. The aim of the study is the targeted metabolomics analysis of 11 modified nucleosides and deoxynucleosides in urine and plasma samples collected from bladder cancer patients with the use of LC-QqQ/MS technique. Since proper sample treatment influences obtained results significantly, the first task of the research covered the development of sample preparation procedure. This included optimization of separation conditions and solid-phase extraction procedure (SPE). Different chromatographic conditions were compared, including: type of stationary phase, flow rate, column temperature and gradient programme. According to SPE, differences in the sugar moiety between nucleosides and deoxynucleosides cause differences in their extraction ability and consequently difficulties with sorbent selection that allows for the extraction of nucleosides and deoxynucleosides at once. Previously, nucleosides and deoxynucleosides were more often analyzed separately. The goal was to develop method for simultaneous extraction of nucleosides and deoxynucleosides from urine and plasma. Different sorbents were evaluated by their selectivity, recovery and ability to extract modified nucleosides and deoxynucleosides. Method based on selected sorbent was further optimized. Acknowledgement: The work has been supported by the National Centre of Science, project no 2018/29/N/NZ7/02299.

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BIOMEDICAL

P-70 Application of targeted metabolic analysis to study glycolysis-related metabolic changes in oral cancer cells

PRESENTING AUTHOR: ***Chul-Ho Jeong, College of Pharmacy, Keimyung University, South Korea***

CO-AUTHORS: ***Won-Jun Jang, Ji Hae Seo***

The application of metabolomics to cancer research field could help to explain the causes of phenotypic changes during acquisition of drug resistance and metabolic alteration in cancer cells. In this reason, understanding metabolic change induced by altered glycolytic pathway in cancer cells is crucial for overcoming drug resistance in various cancers. In the present study, the mass spectrometry-based targeted metabolic profiling was performed to compare the metabolic changes in oral cancer cells, SCC15 following treatment with 2-deoxy-D-glucose (2-DG), 2-DG+pyruvate, and 2DG+mannose. Then we quantified and compared statistically five metabolite groups, acylcarnitines, amino acids and biogenic amines, glycerophospholipids, sphingolipids and monosaccharides. Our targeted metabolomic analyses revealed that significant changes were evident in metabolic pathways involved in the regulation of amino acids (15), polyamine (1), and fatty acids (3). Significant decreases in the levels of amino acids, polyamine and significant increases in the levels of the lysophosphatidylcholine, phosphatidylcholine and sphingomyelin were observed in 2DG-treated SCC15 cells. Furthermore, we found that treatment with pyruvate or mannose significantly restored metabolic changes in 2DG-treated SCC15 cells. Collectively, this study reveals that there are increased metabolic demands reflecting the changes in glycolysis-related amino acids metabolism, which might advance our understanding of metabolic alteration in cancer.

P-71 Metabolic correlations to weight change in patients with head- and neck cancer: A pilot study

PRESENTING AUTHOR: ***Kristian Pirttilä, Uppsala University, Sweden***

CO-AUTHORS: ***Ylva Tiblom Ehrsson, Göran Laurell, Mikael Hedeland, Torbjörn Arvidsson, Curt Pettersson***

Patients afflicted with head and neck cancer (HNC) often suffer from unintentional weight loss and cancer cachexia is prevalent. This pilot study is a part of a multi-center study of HNC patients in Sweden. The aim was to investigate the correlation of the plasma metabolome at different time points to the weight change of patients. Plasma samples from 31 patients taken at three different time points (at diagnosis, 7 weeks after treatment started, and 12 weeks after treatment ended) were analyzed using a HILIC-UHPLC-Q-TOF-MS-protocol. The treatment modality of patients varied, however 27 out of the 31 patients had received radio therapy. The metabolome at the different time points as well as changes between time points were correlated using orthogonal projection to latent structures (OPLS) to a weight outcome factor calculated for each patient based on clinical measurements. A correlation with weight outcome was found in the 7 week (R2 0.807, Q2 0.412, $p = 0.07$) and 12 week follow-up samples (R2 0.679, Q2 0.361, $p < 0.01$) and also in the fold changes from the baseline to the 7 week follow-up (R2 0.722, Q2 0.387, $p < 0.05$). In total 111 metabolites were selected from the models. Among the metabolites with correlation were a number of lysophosphatidylethanolamines (LysoPE) and lysophosphatidylcholines (LysoPC) along with a number of short-chain acyl carnitines. These partial results may suggest downregulation of lipogenesis in the liver in correlation to weight loss, warranting further research.

P-72 Discovery of sex differences in colon cancer metabolism

PRESENTING AUTHOR: ***Yuping Cai, Yale University, United States***

CO-AUTHORS: ***Yuping Cai, Nicholas Rattray, Yawei Zhang, Sajid Khan, Caroline H. Johnson***

Colorectal cancer (CRC) is one of the most common and lethal cancers around the world. While women have a lower incidence of CRC than men they have a higher likelihood of primary tumor presentation on the right-side of colon. This is of concern as epidemiologic studies have shown that patients with right-sided colon cancer (RCC) have poorer clinical outcomes than those with left-sided colon cancers (LCC). However, the reasons for this difference in outcome are not known. Aberrant metabolism is a significant biological feature of CRC; and gene expression patterns have shown that some cancers can be categorized into a “metabolic subtype” of which the abundance is skewed towards RCCs. To unravel sex differences in metabolism for CRC patients, we performed untargeted profiling on colon tissues (normal and tumors; n=271) with stratification by sex and primary tumor location. Our preliminary data shows that metabolites are significantly altered in both women and men with CRC, and are enriched in the following pathways: glycolysis, amino acid metabolism, methionine metabolism, and nucleotide metabolism. Widespread sex-specific differences in metabolites were seen and included tryptophan, citrulline, lactate, N1, N12-diacetylspermine, fatty acids, and fatty acyl carnitines. Of note, when comparing women and men with RCC, women had elevated fatty acid metabolism and transportation, whereas in men, fatty acid utilization was not elevated and instead aerobic glycolysis was upregulated. These results indicate that sex-specific differences exist in tumor substrate utilization for energy and cell growth.

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***AWARD WINNERS**

BIOMEDICAL

P-73 **Metabolomics in rat models of hypertension**

PRESENTING AUTHOR: ***Dorian Maroil, UMONS, Belgium***

Hypertension is a complex and multi-factorial disease and its chronical form affects more than one billion people worldwide. Hypertension may become very critical as it is a major risk factor for cardiovascular morbidity and mortality. Most of hypertension cases are not a consequence of another disease but due to genetic, lifestyle or environmental factors Sometimes, it may also develop after renal failure. Metabolomics is a powerful tool to evaluate global metabolic perturbations in relation with disease, toxicity or treatments. It has proved very helpful for the follow up and identification of early potential biomarkers of these conditions. In order to identify underlying mechanisms involved in the onset of hypertension, we compared the metabolomics signature of either spontaneous or chemically-induced rat models of hypertension. Hypertension was induced in rats by oral administration of NO synthase inhibitor, L-NAME, through the drinking water for 4 weeks. This treatment not only increased the blood pressure but also reduced the renal function. The second model was based on Spontaneously hypertensive (SHR) rats which developed hypertension at the age of 6 weeks. The comparison of metabonomic signatures obtained by proton NMR spectroscopy of urine and serum samples collected from both rat models highlighted some common metabolic patterns which could be further developed as potential biomarkers of this pathology.

P-75 **Statins and antiplatelet treatment after acute myocardial infarction in patients with acute myocardial infarction: HILIC-MS targeted metabolomics of the BATTLE-AMI study**

PRESENTING AUTHOR: ***Carolina Raíssa Costa Picossi, Fundacion San Pablo CEU, Spain***

CO-AUTHORS: ***Carolina Raíssa Costa Picossi, Ana Paula de Godoy Fernandes, Jorge Sáiz, Andréa Tedesco Faccio, Marina Franco Maggi Tavares, Coral Barbas, Francisco Javier Rupérez***

Acute myocardial infarction (AMI) is the largest cause of mortality in the world. The 2017 ESC Guidelines for the management of AMI recommend the administration of an antiplatelet besides the conventional statin therapy although the metabolic consequences of such simultaneous use are not yet fully explored. The objective of this work was to evaluate by a targeted metabolomics approach, part of the metabolic mechanisms involved in 4 combinations of treatments after cardiac ischemia, as part of a comprehensive study within the BATTLE-AMI clinical trial (NCT02428374). Plasma samples were collected from 369 patients who suffered AMI with ST-segment elevation, one day and six months after AMI. All patients received one combined treatment with one antiplatelet (Clopidogrel or Ticagrelor) plus one statin (Simvastatin or Rosuvastatin). After a judicious method development, samples were analyzed by HILIC-MS in positive mode. HILIC provided high separation efficiency of polar analytes. Besides mobile phase and gradient optimization, method development included matrix effect study and data acquisition parameters for Dynamic MRM optimization for 38 compounds including acylcarnitines, amino acids and related compounds. All compounds were quantified, and compared by means of repeated measures ANOVA. Overall, all treatments were equivalent, and significant differences could be seen in less than 25% of the measured compounds, associated either to the combination or the efficiency of the treatment. Lysine metabolism (catabolism and methylation) upraises as the most affected process in these patients. The consequences of such differences will be evaluated in the context of the whole trial.

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***AWARD WINNERS**

BIOMEDICAL

P-77 Estimation of Metabolomic Networks with Gaussian Graphical Models

PRESENTING AUTHOR: ***Katherine Shutta, University of Massachusetts Amherst, United States***

CO-AUTHORS: ***Subhajit Naskar, Kathryn M. Rexrode, Denise M. Scholtens, Raji Balasubramanian***

Network-based metabolomic analyses have high potential to capture signatures of complex biological processes. Topological network properties such as structure, sparsity, and degree distribution provide a systems-based perspective. Unsupervised network-based approaches can provide insights into functional pathways that traditional methods have not elucidated. Estimated networks can provide reinforcement for, or rationale to challenge, previously discovered pathways. The mapping of metabolites to biological pathways is incomplete; network-based approaches may foster advances in the field. Gaussian graphical model (GGM) estimation is one such approach. Modeling metabolomic data with a GGM enables biologically meaningful interpretation of the estimated edge set as a map of functional dependence between metabolites, conditioned on biological state. GGM estimation for high-dimensional datasets is an active area of research. Recently, several open-source R packages have been developed for this purpose. GGM estimation involves several choices with regard to scoring criteria, precision matrix estimation algorithms, and data transformations. For a fixed experimental setting, the different estimation methods generated by these options may result in substantially different GGM estimates. Similarly, the effectiveness of an estimation method at identifying the true network may be highly dependent on experimental factors such as sample size, number of metabolites measured, and network topology. We present results from in-depth simulations designed to characterize these variations, with the goal of providing practical guidance to researchers applying GGM approaches to metabolomics data. We consider various sample sizes, sparsities, and topologies. Application of these approaches is illustrated using data from a cardiovascular disease metabolomics study nested within the CATHGEN repository.

P-78 Untargeted Multiplatform Metabolomics in the quest for biomarkers of cardiac alterations in patients with Cri Du Chat Syndrome

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CO-AUTHORS: ***Fernando Brunale Vilela de Moura Leite, Nilson Antonio Assunção, Coral Barbas, Francisco Javier Rupérez,***

Cri Du Chat Syndrome (CdCS) is a rare genetic syndrome resulting from a partial deletion of the short arm of chromosome 5, which affects an estimated 1 in 50000 live births. Congenital cardiac anomalies in CdCS patients are present in 15 to 20% of the patients. This complication worsens patients' prospect. Detection of specific biomarkers within CdCS patients will help to understand the relationship between cardiac problems and CdCS. Moreover, new diagnostic biomarkers will help in early detection and management of cardiac alterations. Untargeted multiplatform metabolomics (GC/MS, CE/MS and LC/MS – lipidomics) was applied to plasma samples from CdCS patients with (n=13) and without (n=13) cardiopathy. Ion grouping (Molecular Feature Extraction), deconvolution, and multialignment provided the matrices for statistical analysis. Afterwards, multivariate analysis (Principal Components Analysis, Hierarchical Clustering), as well as univariate analysis were performed. 44 statistically significant compounds, differentiating between cardiopathy and control groups were annotated. Level 2 and 3 annotation (MSI) could be achieved by means of different strategies, including CEU Mass Mediator (REF) for simultaneous query in online databases. 25 metabolites increased and 19 decreased in cardiopathy patients. Results suggested dysfunction in fatty acid and phospholipid metabolism, as well as in amino acids and short chain organic acids. Furthermore, a multivariate ROC Curve (AUC=0.974) based on Linear Support Vector Machine showed that 34 metabolites could be used combined as potential biomarkers to diagnose cardiopathy in CdCS patients. The interpretation of these results provides further insight in understanding cardiac diseases in Cri du Chat Syndrome.

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BIOMEDICAL

P-79 Metabolomics Studies Identify Sugar Alcohols as Predictors of Incident Cardiovascular Disease Risks

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CO-AUTHORS: ***Hassan Alamri, Ina Nemet, Nisreen Nimer, Tomas Cajka, Oliver Fiehn, Wilson Tang, Valentin Gogonea, Stanley L. Hazen***

Identification of novel metabolites for early identification of individuals at risk for future cardiovascular events is critical to provide new ways to both diagnose disease states and reveal previously unsuspected pathways associated with cardiovascular disease (CVD) and its pathogenesis. We performed untargeted metabolomics studies employing high resolution gas chromatography mass spectrometry on >12 h fasting plasmas from sequential consenting subjects (n=1162) undergoing elective diagnostic cardiac evaluations with longitudinal follow-up and adjudicated incident (3 year) adverse event monitoring. Among the identified metabolites, several sugar alcohols were significantly associated with incident CVD event risk. To validate the semi-quantitative untargeted metabolomics findings, an independent and new stable-isotope dilution liquid chromatography tandem mass spectrometry method (LC-MS/MS) for the quantitation of sugar alcohols was developed and validated. The method includes plasma protein precipitation and sugar alcohols derivatization with acetic anhydride, followed by liquid-liquid extraction and LC/MS/MS analysis. Preliminary analysis on small and independent validation cohort of subjects (n=200) undergoing cardiac risk factor evaluation/modification in a preventive cardiology clinic replicated the observed CVD risks associated with select sugar alcohols. A complete validation analysis of the targeted sugar alcohols as predictors of incident CVD risks (myocardial infarction, stroke or death) in a large and non-overlapping cohort of patients (n > 2000) also confirmed the potential clinical utility of sugar alcohols as predictors of incident CVD risks. Mechanistic animal model studies to explore whether associations observed between select sugar alcohols confirmed and CVD phenotypes are causally linked are under way.

P-80 Untargeted NMR Metabolomics of Adrenal Hypertension

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CO-AUTHORS: ***Jaap Deinum, Graeme Eisenhofer, Maria-Christina Zennaro, Paolo Mulatero, Alek Prejbisz, Felix Beuschlein, Martin Fassnacht, Livia Lenzini, Michael Conall Dennedy, Marco Boscaro, Filippo Ceccato, Gian Paolo Rossi, JM Connell, Tracy Williams, Michel Azizi, Laurence Amar, Jérôme Bertherat, Anne-Paule Gimenez Roqueplo, Casper K Larsen, Eleanor Davies, Udo FH Engelke, Ron A Wevers, Leo AJ Kluijtmans, Henri JLM Timmers***

Background: The ENSAT-HT project aims to establish a biomarker signature for different forms of adrenal hypertension (AHT) based on a multi-omics approach, including untargeted plasma metabobolomics. Our aim is to define the metabolic signature of AHT patients suffering from either Primary Aldosteronism (PA, n=106), Cushing's Syndrome (CS, n=39) or Pheochromocytoma and Paraganglioma (PPGL, n=98), and compare it to primary hypertensives (PHT, n=109) and healthy volunteers (HV, n=133). Methods: Heparinized plasma samples were collected from all study subjects and analyzed in a randomized order, along with Quality Controls (QC), using proton Nuclear Magnetic Resonance spectroscopy (1H-NMR). The resulting spectra were processed and converted to a readable table by multivariate statistical analysis (MVA) tools; Principal Component Analysis (PCA) was applied to check for outliers and trends within the dataset and Partial Least Squares Discriminant Analysis (PLS-DA) to build a sample classification model. Results: Upon inspection of the PCA score plot of NMR plasma data, a tight clustering of the QC samples was observed, indicating the analytical robustness of the complete methodological approach. A PLS-DA model was built for classification of samples in either PHT or AHT with an accuracy of >80%. Conclusions: A reliable NMR metabolomics approach was applied on human plasma samples. The initial analysis suggests that patient classification based on the presence of either adrenal or primary hypertension appears to be feasible. Future plans include diminishing the effects of confounders and the application of Multilevel MVA methods to delineate the pathophysiological mechanisms of AHT.

P-81 Omics approach to study dyslipidemia

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CO-AUTHORS: ***Lucia Merolle, Chiara Maraccini, Erminia di Bartolomeo, Luca Scarano, Stefania Bergamini, Roberto Baricchi, Thelma Pertinhez***

Dyslipidemia, lipoprotein overproduction or deficiency, is an important risk factor for coronary heart disease and stroke. Metabolomics information about dyslipidemic patients is still limited. Patients non-responder or intolerant to statins usually undergo lipoprotein apheresis (LA) to lower LDL levels. LA exert pleiotropic effects altering a number of blood components. [1,2] We performed a multi-parametric assessment of the inflammatory, PCSK-9 level, together with metabolic and proteomic profiles of dyslipidemic patients undergoing LA to gain information about LA effects and to deeper investigate the disease. Serum samples from 9 patients undergoing three consecutive LA (B. Braun) procedures were assayed. Samples were collected before and after each procedure. Nuclear magnetic resonance and ESI-Q-ToF LC/MS were used to assess metabolomics and proteomic patterns, respectively. Lipid profile and PCSK-9 levels were biochemically measured. Together with the expected depletion of lipoprotein-related proteins and PCSK-9 levels, we observed a reduction of complement- and inflammation-related proteins after each LA procedure. We identified around 50 soluble metabolites defining the metabolic baseline profile of dyslipidemic patients (before LA treatment). LA influences patient's proteomic pattern whereas has no impact on intermediate and final products of metabolic pathways. For 3 out of the 9 patients, we identified the accumulation of branched-chain amino acids. The integration of metabolomics with other omic-based approaches turns out to be relevant to identify clinical markers to support physicians in the choice of the best treatment options.

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*AWARD WINNERS

BIOMEDICAL

P-82 Metabolomics approach coupled with Leaman score to re-stratify coronary artery plaque burden: a translational pilot study with asymptomatic subjects of intermediate cardiovascular risk

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Background: Current risk stratification strategies for coronary artery disease (CAD) have low predictive value in asymptomatic patients classified as intermediate Framingham risk. Metabolomic approach, in addition to detailed anatomical imaging by coronary computed tomography angiography (CCTA), could allow to better identify patients at risk of cardiovascular events. Purpose: To analyze the overall metabolic profile within the tomographic Leaman (CT - LeSc) terciles on the most clinically relevant scenarios - CNvsT3, T1vsT3, CNvsT1. Methods: A total of 20 asymptomatic subjects classified as intermediate CAD risk according to the Framingham score with negative test for ischemia (myocardial perfusion scintigraphy), and normal ventricular fraction (LVEF) were recruited into four groups (CN, T1, T2, T3) according to CCTA results and evaluated by UPLC-QTOF/MS based untargeted metabolomics, correlating to patient's clinical data. CEU Mass Mediator was used to annotate the significant metabolites after univariate (t-test) and multivariate non-supervised analysis for non-parametric data. The lipidic profile was compared with several markers of CAD regarding the capacity to reclassify cardiovascular risk. Results: Within the clinical phenotypes, differential metabolites were highlighted: i) control and higher risk subjects (T3>CN): MGDG, PS (22:0); ii) higher vs lower risk subjects (T3>T1): LysoPI (22:1), DG (34:2), iii) subtle clinical phenotypes (T1>CN): SM (d44:1). Conclusion(s): Metabolomics approach may be useful in clinical practice as a powerful tool for the prediction of CAD in asymptomatic subjects. The tomography Leaman phenotypes showed discriminant metabolite findings as associated with abnormal lipid metabolism and atherosclerosis early formation, enabling a molecular possibility of complementary re-classification of vulnerable subjects.

P-83 Lipidome improves predictive ability of subclinical atherosclerosis over traditional risk factors: The Cardiovascular Risk in Young Finns Study

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CO-AUTHORS: *Pashupati P. Mishra, Leo-Pekka Lyytikäinen, Binisha H. Mishra, Mika Kähönen, Olli T. Raitakari, Reijo Laaksonen, Terho Lehtimäki*

Background: Early detection of atherosclerotic process is crucial for primary prevention of cardiovascular diseases. Subjects and methods: We investigated predictive ability of serum lipidome using LC-MS/MS technique for subclinical atherosclerosis assessed by carotid intima-media thickness (cIMT) in Young Finns Study cohort in 2007 (number:2009, age:30-45 years, women:55%). Statistical analysis was done with dichotomized cIMT data (cases: value >= 90th percentile vs. controls: value <90th percentile). Differential expression analysis of lipidome data between cases and controls was performed with Student's t-test. Differentially expressed lipid species were selected for prediction models . Reference predictive model with major traditional risk factors and two test predictive models; one with lipid species and traditional risk factors (test model1) and other with lipid species only (test model 2) were analyzed. Model fitting and validation was done for 1000 bootstraps of original data by: i) fitting models to training data (70% data), ii) testing the models on test data (30% data), and iii) calculate accuracy measure (ROC AUC). Results: Our results suggest that serum lipidome significantly improves prediction accuracy for subclinical atherosclerosis over traditional risk factors (reference model: AUC 0.762, 95% CI [0.698, 0.823], test model 1: AUC 0.778, 95% CI [0.715, 0.838], t-test p-value: 2.2e-16). Serum lipidome also showed independent predictive accuracy equivalent to that of traditional risk factors [test model 2: AUC 0.762, 95%CI [0.698, 0.821], t-test p-value against reference model: 0.5005]. Conclusion: This study indicates that serum lipidome may help in predicting subclinical atherosclerosis for primary prevention more effectively than traditional risk factors alone.

P-84 Fast LC-ESI-MS/MS analysis and influence of sampling conditions for gut metabolites in plasma and serum

PRESENTING AUTHOR: *Tom van der Laan, Leiden University, Netherlands*

CO-AUTHORS: *Tom van der Laan, Tim Kloots, Marian Beekman, Alida Kindt, Anne-Charlotte Dubbelman, Amy Harms, Cornelia van Duijn, Eline Slagboom, Thomas Hankemeier*

In the past few years, the gut microbiome has been shown to play an important role in various disorders including in particular cardiovascular diseases. Especially the metabolite trimethylamine-N-oxide (TMAO), which is produced by gut microbial metabolism, has repeatedly been associated with an increased risk for cardiovascular events. Here we report a fast liquid chromatography tandem mass spectrometry (LC-MS/MS) method that can analyze the five most important gut metabolites with regards to TMAO in three minutes. Fast liquid chromatography is unconventionally used in this method as an on-line cleanup step to remove the most important ion suppressors leaving the gut metabolites in a cleaned flow through fraction. We compared different bloodmatrix types to recommend best sampling practices and found citrated plasma samples generally demonstrated lower concentrations and choline concentrations were significantly higher in serum samples. We demonstrated the applicability of our method by investigating the effect of a standardized liquid meal (SLM) after overnight fasting of 25 healthy individuals on the gut metabolite levels. Volunteers with increased and decreased gut metabolite serum levels could be identified, which could have been driven by the gender of the volunteers.

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BIOMEDICAL

P-85 Non-targeted metabolomics approach to elucidate metabolism alteration in mice hearts with cardiac-specific Retinoid X Receptor deletion

PRESENTING AUTHOR: ***Francisco Rupérez, Universidad San Pablo-CEU, Spain***

CO-AUTHORS: ***Viviane Aparecida Rodrigues Sant'Anna, Ana Paredes, Miguel Fernández-García, Mercedes Ricote, Coral Barbas***

The nuclear receptor Retinoid X Receptor (RXRs) is a family of ligand-dependent transcription factors that play essential role in heart development. Whereas the systemic RXRα deletion causes embryonic lethality due to cardiac defects, the functional contribution of RXRs to cardiac metabolism remains unknown. To explore such contribution, cardiac-specific RXRs deletion was induced, and the differences on adults and neonate mice hearts metabolism, were evaluated compared to wild-type mice using a non-targeted metabolomics approach with GC-MS and LC-MS. Frozen pieces of adult hearts, and the whole newborn organ (5 mg in average) where homogenized, and from the same homogenate aliquots were processed in parallel for GC-MS(Q) and LC-MS(qTOF) analysis. Raw data from by GC-MS were deconvoluted, aligned and integrated, and metabolites were annotated by comparison of fragmentation spectra with databases. For LC-MS data, molecular recursive feature extraction was performed for ion grouping and signal multialignment. Data matrices were exported for filtration, normalization, scaling and further univariate and multivariate (unsupervised and supervised) statistical analysis. In the case of LC-MS data, only relevant compounds were putatively annotated using CEU Mass Mediator (http://ceumass.eps.uspceu.es/) Results proved that there was a neat difference in the metabolic profile (amino acids, sugars, and lipids) between adults and newborns. Moreover, the different genotypes were strongly associated to the difference in the lipid profile, both in adults and newborns. Differences in the amino acid and central carbon metabolism between adults could be found, but in the newborn mice the profiles were similar in both genotypes.

P-86 Sex-specific metabolic adaptations to diminished nutrient supply

PRESENTING AUTHOR: ***Raphaela Fritsche, Berlin Institute of Health Metabolomics Platform and Max-Delbrück-Center for Molecular Medicine, Germany***

CO-AUTHORS: ***Yoann Gloaguen, Cornelia Bartsch, Angelika Vietzke, Verena Stangl, Mario Lorenz, Jennifer Kirwan***

Underlying molecular mechanisms for sex differences in the pathogenesis of cardiovascular diseases are poorly understood. In addition to hormonal influences, recent findings point to intrinsic sexual dimorphisms at the cellular level. To analyze sex differences in cellular responses to stress, we used male and female human endothelial cells from umbilical cords (HUVECs) from twin pairs. Previously, we had observed a greater ability of female HUVEC cells to form capillary-like structures compared to male cells. To investigate this further, a gas chromatography mass spectrometry (GC-MS) targeted approach was adopted to analyze HUVEC cells raised in different conditions. We focused on key metabolites associated with the central carbon metabolism (CCM). Untreated male HUVECs had a tendency towards slightly higher CCM pools compared to females. In contrast, female cells had altered levels of metabolite pools after serum starvation and VEGF suggesting better stress adaptation. Combined with our earlier findings of increased intracellular ATP levels and higher VEGF- induced migration in female cells, this suggests a higher or more efficient metabolic activity. This is further supported by a higher cell viability of female cells under serum starvation. We propose that female cells have an energetic advantage over male cells under conditions of diminished nutrient supply resulting in a superior ability to cope with nutritional stress and leading to an improvement in translating the VEGF signals into migration. This could have clinical implications for the treatment and prognosis of cardiovascular and other diseases.

P-87 Identification of biomarkers in Attention Deficit Disorder with or without Hyperactivity (ADHD)

PRESENTING AUTHOR: ***Patrick Emond, University of Tours, France***

CO-AUTHORS: ***Camille Dupuy, Patrick Emond, Antoine Lefevre, Sylvie Mavel, Sylvie Bodard, Pierre Castelnau, Laurent Galineau***

Attention Deficit Disorder Hyperactivity Disorder (ADHD) is a heterogeneous neuro-developmental disorder affecting 3-5% of school children and characterized by attention deficit, hyperactivity and impulsivity. Currently the diagnosis is made mainly using cognitive tests with a significant risk of diagnostic errors. Nowadays, no study has found reliable biomarker(s) for ADHD. The identification of biomarkers thus remains an important challenge for early diagnosis and appropriate therapeutic follow-up of patients. Associated with the search for circulating biomarkers, the study of central metabolism is crucial to better understand the pathophysiology of ADHD. As such, the use of animal models is relevant for studying the cerebral metabolome. The best characterized and most used rat model is the SHR / NCrl strain compared to the control strain WKY / NHsd. The objective of this study is to identify metabolic biomarkers, centrally and peripherally, in SHR / NCrl rats. For this, ten brain regions were taken, as well as peripheral samples (blood, urine and faeces), then analyzed in LC-HRMS. The data obtained were processed by multivariate, univariate analyzes and the discriminating metabolic pathways were searched. This study shows a discrimination between the two strains based on their metabolism. The metabolic pathway analysis makes it possible to differentiate two functional networks: the limbic ventral network and the cognitive dorsal network. In addition, the statistically significant alteration of common metabolic pathways is found in brain regions and peripheral compartments. Metabolomics studies on clinical peripheral samples will be carried out to better characterise this severe and frequent disorder.

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*AWARD WINNERS

BIOMEDICAL

P-88 Effect of ASIC3 on metabolomics in serum and urine of fibromyalgia mice

PRESENTING AUTHOR: ***Wei-Hsiang Hsu, China Medical University, Taiwan***

CO-AUTHORS: ***Cheng-Han Lee, Yen-Ming Chao, Chih-Cheng Chen, Yun-Lian Lin***

Fibromyalgia (FM) is a chronic widespread pain. The pathogenesis of FM is unclear. No specific biomarkers are available. Therefore, an animal models of FM may provide an opportunity to explore the potential biomarkers in a relative homogenous disease condition. Here, we probed the metabolomics in an intermittent cold stress (ICS)-induced FM mouse model and focused on the role of acid-sensing ion channel 3 (ASIC3), because ICS treatment induced chronic widespread muscle pain lasting for a month in wild-type (Asic3+/+) mice, but not in Asic3 knockout (Asic3-/-) mice. Serum and urine samples were collected from both genotypes in different ICS stages, including before ICS (basal level, P0), and post-ICS at days 10 (middle phase, P10), and 40 (recovery phase, P40). 1H-NMR- and LC-MS-based metabolomics showed significant difference between control groups and ICS-induced FM groups. Pathway analysis demonstrated that leading regulated pathways in mice were taurine and hypotaurine metabolism, cysteine and methionine metabolism, glycerophospholipid metabolism, and ascorbate and aldarate metabolism. Further, an algorithm was developed for the most impact biomarkers in the FM model. They were cis-aconitate, kynurenate, taurine, pyroglutamic acid, pyrrolidonecarboxylic acid, and 4-methoxyphenylacetic acid in urine, as well as carnitine, deoxycholic acid, lysoPC (16:0), lysoPC (20:3), oleoyl-L-carnitine, and trimethylamine N-oxide in serum. Taken together, these results may serve as potential biomarkers for ASIC3-dependent FM diagnostic or therapeutic targets.

P-89 Enhanced trans-sulfuration metabolically defines a distinct class of ALS patients

PRESENTING AUTHOR: ***Qiuying Chen***

CO-AUTHORS: ***Davinder Sandhu, Csaba Konrad, Dipa Roychoudhury, Benjamin I. Schwartz, Roger R. Cheng, Kirsten Bredvik, Hibiki Kawamata, Elizabeth L. Calder, Lorenz Studer, Steven. M. Fischer, Giovanni Manfredi***

Amyotrophic lateral sclerosis (ALS) is a disease characterized by progressive paralysis and death. Most ALS cases are sporadic (sALS) and patient heterogeneity poses a formidable challenge for developing viable biomarkers and effective therapies. Applying untargeted metabolite profiling on 77 sALS-derived primary dermal fibroblast lines and 45 sex/age matched controls, we found a class of ~25% lines (sALS-1) were characterized by upregulated trans-sulfuration pathway, where methionine-derived homocysteine is channeled to cysteine and glutathione synthesis. sALS-1 fibroblasts exhibit a distinct growth defect in oxidative conditions, which is fully rescued by N-acetylcysteine. [U-13C]-glucose tracing shows that activation of the trans-sulfuration pathway is associated with accelerated glucose flux into the TCA cycle. Based on four metabolites, we developed a support vector machine model capable of distinguishing sALS-1 with 97.5% accuracy. Importantly, plasma metabolite profiling identifies a systemic perturbation of cysteine metabolism as a hallmark of sALS-1. These results indicate that sALS patients can be stratified into distinct metabotypes, differently sensitive to metabolic stress, and provide new insights into metabolic biomarkers and personalized therapies for sALS.

P-90 Tryptophan metabolite profile changes in multiple sclerosis

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CO-AUTHORS: ***Gábor Kecskeméti, Edina Cseh, Elza Szabó, Cecília Rajda, László Vécsei, Tamás Janáky***

Multiple sclerosis (MS) is disabling autoimmune, inflammatory neurodegenerative demyelinating disease affecting the central nervous system. Approximately 2.3 million patients with MS live in the world. Although some changes in tryptophan metabolite profile were associated with MS, we decided to investigate a wider range of metabolites in order to find biomarker(s) for early diagnosis of the disease. A short UPLC-tandem mass spectrometry method was developed for targeted analysis of twelve most important tryptophan metabolites (serotonin - SERO, kynurenine - KYN, 3-hydroxyanthranilic acid - 3-HANA, tryptophan - TRP, 5-hydroxyindoleacetic acid - 5-HIAA, anthranilic acid - ANA, kynurenic acid - KYNA, 3-hydroxykynurenine - 3-HK, xanthurenic acid - XA, melatonin - MELA, picolinic acid - PICA and quinolinic acid - QUIN) in human cerebrospinal fluid. To achieve absolute quantification stable isotope-labeled internal standards (d4-SERO, d4-KYN, d3-3-HANA, d5-TRP, d5-5-HIAA, d5-KYNA, d3-3-HK, d4-XA, d4-MELA, d4-PICA and d3-QUIN) were used. Three metabolites were analysed in derivatized form (3-HK, PICA and QUIN) together with nine underivatized ones. Following the official guidelines, a validation process was carried out to determine the intra- and inter-day precision and accuracy, to confirm the robustness and sensitivity of the developed method. Compared to the control group a dramatic increase of QUIN and decrease of KYNA concentration was observed in the MS group, resulting in a significant rising of QUIN/KYNA ratio. A significant increase in the KYN/TRP ratio was observed in MS patients, too. These results are in harmony with changes detected in human plasma.

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*AWARD WINNERS

BIOMEDICAL

P-91 Pharmacokinetic studies and in-vitro metabolism in HepG2 and HepaRG cell lines of novel modafinil analogue CE-123 by LC-HRMS

PRESENTING AUTHOR: *Iva Cobankovic, University of Vienna, Austria*

CO-AUTHORS: *Iva Cobankovic, Judith Wackerlig, Eva Maria Franschitz, Verena Johler, Carina Müller Predrag Kalaba, Gyertyán István, Gábor Brenner, Ernst Urban, Thierry Langer, Gert Lubec*

Modafinil is a wake-promoting drug that has been used for the treatment of excessive sleepiness in patients with narcolepsy. There are several suggested hypotheses of Modafinil mechanism but most probably one is binding of Modafinil to the dopamine transporter (DAT). However, specificity of Modafinil for DAT is limited as it also binds to the serotonin and noradrenaline transporters. Recently, we synthetized a novel Modafinil analogue CE-123 with higher specificity for the DAT and tested its pharmacokinetic properties on aged rats and its metabolism in liver cancer cell lines (HepG2 and HepaRG). Plasma samples from aged rats were taken 2,5 hours after administration of 10 mg/kg CE-123 via intraperitoneal injection. In order to determine the concentration of CE-123, a LC-HRMS method was developed and validated applying Modafinil as internal standard. LOD and LOQ were found to be 2 and 6 ng/mL, respectively. The analyte concentrations were in a range of 1.2-4.1 µg/mL with a mean of 3.1 µg/mL (%CV = 3.20). In our previous study on young rats plasma concentrations after 1 hour of administration of 10 mg/kg CE-123 were 2.3 ± 1.0 µg/mL. The higher concentrations of CE-123 in aged rats could be because of slower metabolism of aged animals. Biotransformation of CE-123 was observed in HepG2 and HepaRG cell lines. After 24 h incubation of HepG2 and HepaRG with CE-123 concentration of analyte decreased for 14% and 3,6%, respectively. An accurate and precise validated analytical method was developed enabling to observe pharmacokinetic properties and biotransformation of novel Modafinil analouges.

P-92 LC-MS Untargeted Analysis in Posttraumatic Stress Disorder

PRESENTING AUTHOR: *Marcela Konjevod, Rudjer Boskovic Institute, Division of Molecular Medicine, Laboratory of Molecular Neuropsychiatry, Croatia (Hrvatska)*

CO-AUTHORS: *Jorge Saiz, Matea Nikolac Perkovic, Gordana Nedic Erjavec, Dubravka Svob Strac, Lucija Tudor, Neven Zarkovic, Coral Barbas, Nela Pivac*

Posttraumatic stress disorder (PTSD) is a mental disorder that often develops after traumatic experience. It is a serious neuropsychiatric disorder characterized with impairments in feeling, thinking, sleeping and normal functioning. Molecular and biological basis of PTSD development and progression is still unclear. The aim of this study was to determine metabolites as possible biomarkers that differ between PTSD subjects and healthy controls. LC-MS (Q-TOF) was used for the analysis of the plasma samples of patients enrolled in this study (50 PTSD subjects and 50 healthy controls) in an untargeted analysis. Sample preparation included protein precipitation with cold methanol : ethanol (1:1). Prepared samples were injected randomly with injection volume set to 10 µL at temperature of 4°C, while flow was set up at 0.6 mL/min. The analysis were performed in positive and negative ionization. The chromatograms were processed and tandem mass spectrometry was performed for compounds statistically significant ($p<0.05$) in order to identify them. Compounds that were tentatively annotated or identified according to the MS / MS analysis mostly belong to the compound group of glycerophospholipids, with glycerophosphocholines as the most common class among glycerophospholipids. Altered levels of glycerophospholipids indicate increased inflammation and impairments associated with cell membrane. Posttraumatic stress disorder is a complex anxiety disorder that affects the whole organism, therefore future studies are necessary to determine possible biomarkers or metabolic pathways associated with PTSD. In order to validate the compounds obtained in this study, the next step will include targeted analysis on a larger number of samples.

P-93 Clinical metabolomics - Similarities and differences between multiple sclerosis and Huntington's disease

PRESENTING AUTHOR: *Stephanie Herman, Uppsala University, Sweden*

CO-AUTHORS: *Payam Emami Khoonsari, Valter Niemelä, Torbjörn Åkerfeldt, Ola Spjuth, Joachim Burman, Kim Kultima*

The metabolome is the set of all metabolites present in a cell, tissue or body fluid in a given time point. It comprises all substrates and end products in the biochemical networks in our body and as it is the closest omics layer to phenotype, it is directly affected by any pathophysiological changes. As such, the metabolome is an excellent compartment to study diversions from the normal state. We have measured the metabolome of cerebrospinal fluid (CSF) from patients in different stages of multiple sclerosis and Huntington's disease as well as in control individuals. Both disease metabolomes were significantly different from the metabolome of control individuals, with area under the receiver operating characteristic (AUROC) curves of 0.79(±0.21) and 0.72(±0.14) for multiple sclerosis and Huntington's disease respectively, and demonstrated significant differences between disease stages (AUROC values of 0.83(±0.15) and 0.76(±0.17), respectively). Inter-group comparisons revealed multiple biochemical pathways that were being altered, including the tyrosine metabolism in Huntington's disease, and the tryptophan metabolism and pantothenate and CoA biosynthesis in multiple sclerosis. In common, the phenylalanine and the nitrogen metabolism were altered in both multiple sclerosis and Huntington's disease. Metabolomics is still a maturing field, which we believe has the potential of developing into something clinically useful. In this study, the metabolic signature of CSF was able to distinguish two neurological diseases from normal state as well as demonstrated differences between stages of the diseases.

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BIOMEDICAL

P-94 First-episode psychotic patients who gain weight in follow-up have increased markers of non-alcoholic fatty liver disease at baseline

PRESENTING AUTHOR: ***Matej Oresic, Örebro University, Sweden***

CO-AUTHORS: ***Santosh Lamichhane, Alex Dickens, Cecilia Carlsson, Jaana Suvisaari, Oliver Howes, Jarmo Hietala, Tuulia Hyötyläinen***

Schizophrenia is associated with a life expectancy reduction of 15-20 years, mostly due to the high prevalence of cardiovascular disease, type 2 diabetes and metabolic syndrome. Identification of psychotic individuals who are at highest risk of rapid weight gain and the associated development of metabolic co-morbidities is therefore a major challenge for public health. We have previously shown in a first-episode psychosis (FEP) prospective study in Uusimaa district in Finland (1) that those FEP patients who rapidly gained weight, had increased circulating lipids that were previously reported as biomarkers of non-alcoholic fatty liver disease (NAFLD) (2). Here we applied lipidomics in a prospective study comprising 48 controls, 44 FEP patients and 22 individuals at clinical-high-risk (CHR) for psychosis, from two study centres (Turku/Finland and London/UK). Lipidomics (UHPLC-MS) was applied in baseline serum samples, while body mass index (BMI) was assessed at baseline and after 12 months. Similarly as in previous study (1), we found that FEP patients who gained weight had at baseline, independent of obesity, significant increase of triacylglycerols with low double bond count and carbon number, as well as decreased phospholipids containing polyunsaturated fatty acids. Our study thus confirms that lipidomic signature of NAFLD at baseline, independent of obesity, may serve as a predictor of weight gain and may thus provide a useful marker for identifying patients who are most vulnerable to the development of metabolic co-morbidities in psychosis.

P-95 Lipid profiles of first episode psychosis

PRESENTING AUTHOR: ***Alex Dickens, University of Turku, Finland***

CO-AUTHORS: ***Santosh Lamichhane, Laurikainen H, Borgan F, Suvisaari J, Howes O, Hietala J, Hyötyläinen T, Matej Orešič***

The metabolic co-morbidities in psychosis are well recognised. The underlying mechanisms for these metabolic changes remain unclear. Here we studied the circulating lipid profiles in 4 independent psychosis cohorts and controls. Two of the cohorts were from Finland (Turku and THL), one cohort from London (KCL) and one from Spain (SERMAS). Our aim was to identify changes in lipids in first episode psychosis (FEP) individuals compared to the controls. The lipids were detected using a UHPLC-QTOF (Agilent 6540) profiling method. The concentrations of the lipids were calculated using class based internal standards and standard curves. The Turku and KCL samples were run in the same batch and used as a discovery cohort (n=57). The THL (n=38) and SERMAS (n=130) were used as independent validation cohorts. The lipid profiles showed a clear effect of site between the Turku and KCL groups. The Gaussian clusters revealed that the main lipids driving this separation were triglycerides suggesting a diet effect in these two populations. There was also a significant effect of gender in the lipid clusters and therefore, we decided to model the genders separately. In the male FEP patients 4 lipids were identified by univariate and multivariate analysis. The 4 lipids were PC (40:5), PC(40:4), PC(32:1), PC(38:6) and all decreased in concentration in FEP. Meta-analysis across all 4 cohorts reveal that these lipids are regulated similarly across the cohorts. These lipid species identified by this study can demonstrate a unique lipid signature for the onset of FEP in a multi-centre setting.

P-96 Analysis of ceramides and sphingosine 1 phosphate in ischemic stroke patients using ultra-high-pressure liquid chromatography - electrospray ionization tandem mass spectrometry

PRESENTING AUTHOR: ***Tsung-Heng Lee, School of Pharmacy, National Taiwan University, Taiwan***

CO-AUTHORS: ***Sung-Chun Tang, Hsi-Chun Chao, Ching-Hua Lee, Ching-Hua Kuo***

Sphingolipids are major constituents in eukaryotic cell membrane and play many key roles in cellular regulation processes, which is also involved in pathophysiological mechanism of many diseases. Increasing evidence has shown that the sphingolipids involved in the development of ischemic stroke and the sphingolipid pathway may act as the potential therapeutic targets. To provide accurate sphingolipid quantification results, we have optimized the sample extraction protocol and the ultra-high-pressure liquid chromatography - electrospray ionization tandem mass spectrometry (UHPLC-ESI-MS/MS) analytical method. To investigate the prognostic roles of ceramide in patients with acute ischemic stroke, we collected plasma samples from acute ischemic stroke patients at 24 hr and 72 hr post stroke (n=95; poor prognosis: 46%) and non-stroke controls (n=55). Plasma samples were measured by the optimized UHPLC-ESI-MS/MS method. The post-stroke outcome was determined by the modified Rankin Scale (mRS), and mRS 2 at 3 months post stroke was defined as a good outcome. The preliminary results show that the levels of sphingosine-1-phosphate in patients significantly decrease compared to the control group. The comparison of ceramides with different acyl chains between patients with different outcomes at 72 hours revealed a significant increase of long-chain ceramide levels, C16, C18, C20, and C22 in patients with poor prognosis. These results suggest long chain ceramides represented as potential prognostic markers for patients with acute ischemic stroke. The biological mechanism of long chain ceramides in ischemic stroke deserves further investigation to elucidate their role as the therapeutic target.

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BIOMEDICAL

P-97 Profiling metabolites in rat brain microdialysates by capillary electrophoresis-mass spectrometry using direct sample injection

PRESENTING AUTHOR: ***Marlien van Mever, Leiden University, Netherlands***

CO-AUTHORS: ***Thomas Hankemeier, Rawi Ramautar.***

Analysis of low-molecular-weight biomolecules in brain extracellular fluid plays an important role in the prediction, progression, and treatment outcome for various neurological diseases. Microdialysis is a powerful technique for in vivo sampling of extracellular fluid in the brain, thereby enabling the continuous measurement of neurotransmitters in freely-moving animals. However, metabolic analysis of microdialysis samples can be considered a huge analytical challenge due to limited sample amounts and low basal (nanomolar-range) concentrations of many metabolites. In this study, we have developed a capillary electrophoresis-mass spectrometry (CE-MS) method for the profiling of amino acids and related compounds in rat brain microdialysates using direct sample injection. CE was coupled to time-of-flight mass spectrometry (TOF-MS) employing a co-axial sheath-liquid interface and a bare fused-silica capillary. Amino acids were analyzed at low-pH separation conditions using 10% acetic acid (pH 2.2) as separation buffer. To increase the concentration sensitivity of CE-MS, an in-capillary preconcentration procedure was applied, which allowed large volume sample injections. The developed CE-MS system allowed the direct analysis of amino acids in rat brain microdialysis samples after only applying a centrifugation step. Low nanomolar detection limits could be obtained for the compounds with minimal sample matrix effects. Overall, we propose here the first CE-MS method for the direct profiling of amino acids and related compounds in rat brain microdialysate samples. The utility of this approach will be tested by analyzing a large set of rat brain microdialysate samples in a pharmacokinetic and biomarker discovery context using metabolomics.

P-98 Impact of preservation conditions on the fecal metabolome and lipidome

PRESENTING AUTHOR: ***Margot De Spiegeleer, Ghent University, Belgium***

CO-AUTHORS: ***Steve Huysman, Lieven Van Meulebroek, Lynn Vanhaecke***

Eminent attention towards fecal fingerprinting is emerging, in that it may unravel the symbiotic interplay between the host, diet and intestinal ecosystem and yield characteristic signatures of responsive metabolic discrepancies regarding gut (patho)physiology and beyond. However, it is instrumental to capture and conserve a realistic snapshot that is reflective for the dynamic metabolome covered upon (outpatient) sampling to circumvent erroneous interpretations and subsequent deceptive associations regarding the disease and parameter(s) under observation. Hence, this study tackles the present-day lack of preservation guidelines regarding the stability of the fecal polar metabolome and lipidome, using in-house developed and validated extraction and UHPLC-HR-Q-Orbitrap-MS methods (Vanden Bussche et al., 2015 & Van Meulebroek et al., 2017, Anal. Chem.). By means of targeted profiling (n>400) and untargeted fingerprinting in concert with univariate and multivariate data analysis, the effect(s) of freeze-thawing (up to 2 cycles) and storage duration (up to 25 weeks), integrated with storage temperature (-20°C and -80°C) and (an)aerobicity were assessed. For the polar metabolome fraction, the impact of aerobicity was concluded to be negligible, which was ascribed to lyophilization of the fecal material. Conversely, freeze-thawing was pointed out to significantly affect metabolome composition with the first cycle being predominant. For example, elevated concentration levels were observed for amines and amino acids. Monitoring the long-term stability, particularly the low-molecular-weight metabolites (<400 Da) exhibited significant alterations during the timecourse of storage, whereby our recommendation sounds to store lyophilized fecal samples no longer than 4 weeks at -20°C and 8 weeks at -80°C.

P-99 Nuclear Magnetic Resonance Urine Metabonomics of Healthy Taiwanese Subjects from the Taiwan Biobank

PRESENTING AUTHOR: ***Chung-ke Chang, Taiwan Biobank, Taiwan***

Human urine is one of the most accessible and information-rich biofluids for metabonomic studies. Many studies have been conducted on the correlation between urine metabonomics and various diseases, but there is little information on the characteristics of the urine metabolome of “healthy” subjects from distinct ethnicities. Establishing such an ethnic “reference metabonome” is necessary for the development of metabonomic applications in a population-wide precision medicine context. Nuclear magnetic resonance (NMR) spectroscopy is especially suited to the task because of its easy sample handling and excellent reproducibility. We hand-picked a set of healthy and fit individuals from participants enrolled in the Taiwan Biobank project and obtained a preliminary reference metabonome NMR spectrum representing ethnic Han Chinese residing in Taiwan. We highlight spectral regions with low variance across the samples, which may represent a population fingerprint of health for Taiwanese individuals. We also compare our findings with results from other population cohort urine metabonomics initiatives.

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BIOMEDICAL

P-100 Metabolomic biomakers of smoking habit in a large population study in Japan: Tsuruoka Metabolomics Cohort Study

PRESENTING AUTHOR: ***Sei Harada, Keio University, Japan***

CO-AUTHORS: ***Ayako Kurihara, Mizuki Sata, Minako Matsumoto, Suzuka Kato, Takuma Shibuki, Miho Iida, Ayano Takeuchi, Akiyoshi Hirayama, Masahiro Sugimoto, Tomoyoshi Soga, Masaru Tomita, Tomonori Okamura, Toru Takebayashi***

[Background and Aim] Smoking causes various health impairment. Recently, new tobacco products such as e-cigarette and heat-not-burn tobacco are becoming popular, but their chronic health effects are not well known. To establish novel methods to evaluate effects by these new products is demanded. Metabolomics is one of promising approaches to find biomarkers of health effects by smoking habit. Therefore, we examined metabolomic biomarkers of smoking effects in a large population-based cohort in Japan. [Methods and Results] We conducted metabolomic profiling by capillary electrophoresis-mass spectrometry (CE-MS) for 10,933 fasting plasma samples of community dwelling participants of the Tsuruoka Metabolomics Cohort Study in Japan. Ninety-four polar metabolite concentrations were absolutely measured. After excluding participants with history of cancer, stroke and ischemic heart disease, 4,573 males and 5,338 females (35-74 years old) were included in this study. Linear mixed model showed that 48 metabolites in males and 28 in females were different between current and never smokers after adjusting age and other confounders as fixed effect and metabolome analysis batch as random effect (FDR-p <0.05). Twenty-two metabolites were common biomarker candidates for both genders. Past smokers had intermediate profile between current and never smokers. Two of these metabolites had clear dose-dependent associations with smoking numbers per day among current smokers. Also, two metabolite concentrations were associated with period after smoking cessation. [Conclusion] Our CE-MS metabolomics platform discovered biomarker candidates related to smoking habit. Found dose and time dependent association suggests that these biomarkers are reliable to evaluate chronic health effect by smoking.

P-101 Metabolomic approach to investigate alteration in metabolites associated with 25-hydroxyvitamin D in healthy Korean adults

PRESENTING AUTHOR: ***Mi-Ri Gwon, Kyungpook National University, South Korea***

CO-AUTHORS: ***Bo Kyung Kim, Seungil Cho, Sook Jin Seong, Young-Ran Yoon***

Vitamin D3 (cholecalciferol) deficiency is prevalent in Korean and affects up to 80% of Korean adults. This vitamin is important to avoid the risk of other health problems including chronic autoimmune, infectious diseases, cancer, cardiovascular disease, and diabetes. Most previous studies focused on finding intermediate metabolites of vitamin D3. To understand the metabolic pathway of vitamin D3, it is important to investigate its association with endogenous metabolites in elderly or osteoporosis-risk groups. Thus, we examined the association between serum level of primary metabolite, 25(OH)D and metabolite alteration in healthy Korean adults for preliminary study. Forty-five healthy Korean adults were divided into two groups based on average serum levels of 25(OH)D: high (n=21, 23.0 ng/mL) and low (n=24, 10.4 ng/mL) groups. Demographic, anthropometric, and blood profile parameters were measured. Metabolic profiling was performed using UHPLC-QTOF/MS and multivariate statistical analysis. The altered metabolites were determined and identified using MetaboScape software. No significant differences were observed in demographic, anthropometric, and blood profile parameters between two groups. After preprocessing UHPLC-QTOF/MS data, we obtained 55,940 spectral features for untargeted metabolomic analysis. Score plots of both principal component analysis and orthogonal projections to latent structures-discriminant analysis showed a tendency for two groups to be separated. In this analysis, R2 (goodness of fit) and Q2 (predictability) were 0.280 and 0.234, respectively. Five endogenous metabolites were finally identified. Further research with larger sample sizes and other clinical trial designs (e.g., administration of 25(OH)D to osteoporotic or elderly people) is required before a solid conclusion can be reached.

P-102 Metabolomic Profiles Associated with Body Mass Index, Waist Circumference, and Diabetes and Inflammation Biomarkers in Older Adults

PRESENTING AUTHOR: ***Victoria Stevens, American Cancer Society, United States***

CO-AUTHORS: ***Brian D. Carter, Marjorie M. McCullough, Peter T. Campbell, Ying Wang***

Metabolomic profiling has been used to identify metabolites altered in overweight and obese individuals but the roles of these metabolites in the development of obesity-related diseases is unclear. To address this, an untargeted, mass spectroscopy-based metabolomic analysis was conducted using serum from 1,534 women and plasma from 554 men aged 53-86 years. Linear regression was first used to identify metabolites associated with body mass index (BMI) and waist circumference (WC) in women and men separately. Next, among these metabolites, blocks of clustered metabolites whose association with the anthropometric measures could be represented by a single metabolite were defined. The association of these representative metabolites with biomarkers for diabetes and inflammation was then determined. Statistically significant associations with BMI and WC were observed both for previously identified metabolites and novel metabolites, including several sphingolipids, nucleotides, and modified fatty acids. Identification of clustered blocks reduced the number of metabolites associated with BMI in women from 260 to 115 and, in men, from 79 to 22. Specific adiposity-associated metabolites were also associated with clinical biomarkers, including a choline-containing plasmalogen (O-16:0/18:1) with c-peptide and adiponectin and glycine with c-reactive protein, suggesting they might contribute to insulin regulation and resistance and inflammation. These results add to the list of metabolites associated with adiposity and indicate which may influence processes that contribute to the development of obesity-related diseases. In addition, the identification of clusters of metabolites using a correlation-based method indicated common chemical features that may help explain their association with the anthropometric measures.

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BIOMEDICAL

P-103 Is there a metabolomic signature indicative of human urine specimen degradation? A systematic review

PRESENTING AUTHOR: ***Ana Rosen Vollmar, Yale School of Public Health, United States***

CO-AUTHORS: ***Caroline H. Johnson***

With metabolomics increasingly used to investigate the exposome, interest is growing in using biobanked samples and other specimens in long-term storage. Specimen quality can vary due to handling procedures, storage conditions, and freeze-thaw cycles. We conducted the first systematic review of whether there is a metabolomic signature of human urine specimen degradation. Using controlled vocabulary terms and text words, studies were identified through PubMed and Web of Science. Seventeen studies met the inclusion criteria of using human urine, and comparing conditions of storage time, temperature, and freeze-thaw cycles. We developed a study quality rating system based on the Metabolomics Standards Initiative. Study quality varied but was generally moderate to high, lending support to our findings. While targeted analyses found significant metabolite changes across comparison groups, untargeted analyses found no meaningful changes in the global metabolomic profile, with biological variability the primary source of variability between specimens rather than handling and storage conditions. More than 2-3 freeze-thaw cycles may alter metabolite composition in targeted studies, but untargeted analyses are robust to even 9 freeze-thaw cycles. Minimal difference was observed between specimens stored at -20 °C and -80 °C. Specimens remained stable for 6 months at -25 °C or -80 °C, but no studies examined storage longer than 6 months. A total of 90 metabolites associated with handling and storage conditions were identified across all studies, with 19 identified by at least 2 studies, indicating there is not yet a clear metabolomic signature of urine degradation, but offering directions for future research.

P-104 Sugars and derivatives in the human metabolome: what they can tell us

PRESENTING AUTHOR: ***Carina Mack, Max Rubner-Institut, Germany***

CO-AUTHORS: ***Christoph H. Weinert, Hannelore Daniel, Sabine E. Kulling***

Sugar compounds (mono- and disaccharides, polyols and sugar acids) are part of the metabolome. Although numerous sugar compounds occur in nature, mostly only a very few common and well-known compounds are analyzed. Metabolomics often requires a compromise between detecting as many different metabolites and substance classes as possible, and satisfactory separation of compounds within each substance class. Sugars with their high structural similarity present a particular challenge with usually insufficient chromatographic and mass spectrometric separation. More comprehensive and highly selective methods to assess the diversity of the human body fluid sugar profile are thus needed because sugar compounds may serve as markers of dietary intake and may act as reporter molecules of the health status. We developed a semitargeted GC-MS based profiling method enabling detection of known and unknown sugar compounds in urine and plasma. 24 h urine samples of the observational Karlsruhe Metabolomics and Nutrition study with 300 healthy participants were analyzed and markers for dietary intake were identified amongst the sugars, such as mannoheptulose and perseitol for avocado consumption. In an additional intervention study including an oral glucose tolerance test, plasma samples of healthy, prediabetic and diabetic participants were analyzed and revealed, next to glucose, a variety of sugars and derivatives with marked postprandial differences dependent on health status, such as trehalose. Overall, the application of the sugar profiling in these human studies revealed a more complex sugar profile than described or expected so far with potential for finding novel markers.

P-105 Metabolic phenotyping of individuals in baseline and repeat surveys of Tohoku Medical Megabank Cohort Project

PRESENTING AUTHOR: ***Seizo Koshiba, Tohoku University, Japan***

CO-AUTHORS: ***Daisuke Saigusa, Ikuko Motoike, Jin Inoue, Eiji Hishinuma, Matsuyuki Shirota, Yuichi Aoki, Shu Tadaka, Kengo Kinoshita, Masayuki Yamamoto***

Tohoku Medical Megabank (TMM) Project operates prospective cohort studies for more than 150,000 individuals in Japan. We have finished baseline surveys and are now conducting repeat surveys. We are also operating genome and omics analyses for the cohort participants and have already collected whole genome sequences data from more than 5,000 participants and the metabolome data from more than 10,000 participants. We have released these results as a database, Japanese Multi Omics Reference Panel (jMorp), which is freely available on the web site (https://jmorp.megabank.tohoku.ac.jp/). In this conference, we report recent progress of our metabolome analyses in the TMM project. We expanded our metabolome analysis and will release NMR metabolome data from more than 15,000 participants at the baseline survey this year. We also quantified more than 1,000 participant's plasma metabolites by means of LC-MS/MS and will also release these results. On the other hand, we have performed metabolome analysis for participants at the repeat assessment survey and will show the changes of metabolic phenotypes of individuals between the baseline and repeat surveys. We are also performing metabolome genome-wide association study (MGWAS) and other association studies. We suggest the importance of the long-term examination of metabolome analysis for the large scale prospective cohort studies.

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BIOMEDICAL

P-106 Metabolomic Amino Acids Profiles as Biomarkers to Identify Metabolic Alterations

PRESENTING AUTHOR: *Dirce Maria Marchioni, University of São Paulo, Brazil*

CO-AUTHORS: *Dirce Maria Marchioni, Regina Mara Fisberg, Alexsandro Macedo Silva*

Background. Metabolic alterations compromises physiological functions, triggering morbidities for humans. Therefore, would be valued to explore biomarkers to recognise metabolic changes using the metabolomics tools. Objective. This study aimed to investigate amino acids (AA) as biomarkers to classify metabolic variations. Methods. Blood samples were obtained from 168 adults (≥20 years) who participated in the population based cross-sectional study "Health Survey of Sao Paulo". The targeted AA metabolome profiling was analysed by HPLC/MS methods. Principal Component Factor Analysis (PCFA) with varimax orthogonal rotation was performed to generate the AA patterns. Obesity was determined based on Body Mass Index ≥30 kg/m^2. HOMA-IR was estimated, considering the cut-off ≥2.7 for insulin resistance (IR) classification. Metabolic Syndrome (MS) was characterized by 3 or more health abnormalities parameters, such as medicine use, blood pressure (≥130mmHg/85mmHg), fasting glucose (≥110mg/dL), triglycerides (≥150mg/dL), HDL-cholesterol (≤40mg/dL for men; ≤50mg/dL for women), and waist circumference (≥102cm for men, ≥88cm for women). The dyslipidaemia (DL) variable was considered one or more factors as followed: LDL-cholesterol (≥160mg/dL); Total Cholesterol (≥190mg/dL); triglycerides (≥175mg/dL). The ability of the AA to discriminate the outcomes was assessed by using area under the receiver operating characteristic curve (AUC). Results. The PCFA produced 5 profiles (P), but only the P1 (Leu, Val, Ile, Phe, Tyr, Trp, Lys, Met, His, Glu, Gly, Asn, Ala), which represented the β-catabolism of AA and lipids showed an promising outcome classification: IR (AUC=0,604); MS (AUC=0,657); Obesity (AUC=0.744); DL (AUC=0.622). Conclusion. The AA pattern revealed a noteworthy characteristic as biomarker for metabolic changes.

P-107 Effects of chronic hypervitaminosis A on global plasma metabolome changes in pig and human

PRESENTING AUTHOR: *Warwick Dunn, University of Birmingham, United Kingdom*

CO-AUTHORS: *Georg Lietz, Anthony Oxley, Kieran Finney, Adam Clark, Tim Giles, Neil Foster, Andrew Southam, Andris Jankevics, Gavin Lloyd, Catherine Winder, Elliot Palmer*

Objectives: Significant concerns about inadvertent chronic excessive vitamin A (VA) intakes due to overly frequent supplementation, fortification and voluntarily fortified products have been raised with primary impact being observed in the Developing World. Clinically detectable signs of VA toxicity are rare, indicating the need for early biomarkers of tissue damage induced by excessive VA intake. Here we will present two studies in pigs and humans. Methods and results: Study 1: To identify early putative markers of VA toxicity, we induced chronic hypervitaminosis A in pigs (64 pigs, 8 per group) dosed with an oral supplement of retinyl propionate (0 up to 10,000µg/KgBW) for 17 weeks. Two untargeted UPLC-MS assays (HILIC and C18 reversed phase) were applied to analyse plasma and urine metabolites followed by univariate and multivariate analysis. Multiple metabolite classes were perturbed primarily focused on lipid metabolism, including (lyso)glycerophospholipids, ceramides and acyl glycerides. Study 2: To identify markers of vitamin A stress we undertook two independent human studies) in Guatemala and the Phillipines (n>150 subjects in each study). Serum and dried serum spots were collected from children and one untargeted UPLC-MS assays (C18 reversed phase) were applied to analyse plasma and urine metabolites followed by univariate and multivariate analysis. Markers indicative of increased blood retinol levels included (lyso) glycerophospholipids, ceramides and acyl glycerides as was observed for the pig study. Conclusions: Potential markers of vitamin A levels and hypervitaminosis A have been identified in a pig model and validated in two independent human studies.

P-108 Chronic alcohol consumption causes dysregulation of the bile acid biosynthesis pathway

PRESENTING AUTHOR: *Georgia Charkoftaki, Yale School of Public Health, United States*

CO-AUTHORS: *Georgia Charkoftaki, Wan Ying Tan, Jaya Prakash Golla, Pablo Berrios-Carcamo, David Orlicky, Vasilis Vasiliou*

Alcohol consumption leads to tissue damage. However, the molecular mechanisms contributing to the damage remain to be fully understood. Our goal was to identify pathways affected in the liver caused by chronic alcohol consumption. Eight – 10 wk old male C57BL/6J mice were fed a modified ethanol-containing liquid diet Lieber-DeCarli (LD) (ethanol-treated, n=4) or an ethanol-free LD diet (control, n=5) for 6 wks. Mice on the ethanol diet started at 2% (v/v) and ethanol was increased 1% weekly over 5 wks, until 6% (v/v) was applied. At the end of the sixth week, the livers collected underwent blinded histological examination and untargeted metabolomic analyses. Multivariate analyses determined differences in the metabolic profiles between ethanol-treated and control mice. Targeted metabolomic analyses was subsequently performed to quantify significant features uncovered in the untargeted metabolomic analyses. Histological examination revealed no inflammatory or injury differences; however, enlarged bile ducts were observed in the ethanol-treated mice. The most prominent change in the metabolic profile of ethanol-treated mice occurred in the bile acid biosynthesis pathway. Utilizing targeted metabolomics, we quantified 19 bile acids. Of these, significant ($P<0.05$) increases in the primary bile acid cholic acid (5.0-fold), and its taurine- and glycine- conjugated bile acids, including taurodeoxycholic acid (2.0-fold), taurohyodeoxycholic acid (3.0-fold), glycocholic acid (11.5-fold) and glycochenodeoxycholic acid (4.8-fold) were observed in the ethanol-treated mice. Our study provides new insights into hepatic changes occurring early during chronic alcohol consumption. How the dysregulated bile acid pathway may contribute to liver damage remains to be established.

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*AWARD WINNERS

BIOMEDICAL

P-109 Patterns in metabolite profile are associated with risk of more aggressive prostate cancer: a prospective study of 3057 matched case-control sets from EPIC

PRESENTING AUTHOR: ***Julie Schmidt, University of Oxford, United Kingdom***

CO-AUTHORS: ***Georgina K. Fensom, Sabina Rinaldi, Augustin Scalbert, Paul N. Appleby, David Achaintre, Audrey Gicquiau, Marc J. Gunter, Pietro Ferrari, Aurora Perez-Cornago, Timothy J. Key, Ruth C. Travis***

Metabolomics may reveal novel insights into the aetiology of prostate cancer, for which few risk factors are established. We investigated the association between patterns in baseline plasma metabolite profile and subsequent prostate cancer risk, using data from 3057 matched case-control sets from the European Prospective Investigation into Cancer and Nutrition (EPIC). We measured 119 metabolite concentrations in plasma samples, collected on average 9.4 years before diagnosis, by mass spectrometry (AbsoluteIDQ p180 Kit, Biocrates Life Sciences AG). Metabolite patterns were identified using treelet transform, a statistical method for identification of groups of correlated metabolites. Associations of metabolite patterns with prostate cancer risk (OR[1SD]) were estimated by conditional logistic regression. Supplementary analyses were conducted for metabolite patterns derived using principal component analysis and for individual metabolites. Men with metabolite profiles characterised by higher concentrations of either phosphatidylcholines and hydroxysphingomyelins (OR[1SD]=0.77, 95%CI 0.66-0.89), acylcarnitines C18:1 and C18:2, glutamate, ornithine and taurine (OR[1SD]=0.72, 0.57-0.90), or lysophosphatidylcholines (OR[1SD]=0.81, 0.69-0.95) had lower risk of advanced stage prostate cancer at diagnosis, with no evidence of heterogeneity by follow-up time. Similar associations were observed for the two former patterns with aggressive disease risk (the more aggressive subset of advanced stage), while the latter pattern was inversely related to risk of prostate cancer death (OR[1SD]=0.77, 0.61-0.96). No associations were observed for prostate cancer overall or less aggressive tumour subtypes. In conclusion, metabolite patterns may be related to lower risk of more aggressive prostate tumours and prostate cancer death, and might be relevant to aetiology of advanced stage prostate cancer.

P-110 Metabolic profiling of mice cerebral tissue for the study of acute and chronic ethanol toxicity

PRESENTING AUTHOR: ***Emily Armitage, Shimadzu Corporation, United Kingdom***

CO-AUTHORS: ***Helen Gika, Olga Deda, Christina Virgiliou, Georgios Theodoridis, Ian Wilson, Stephane Moreau, Neil Loftus***

Alcoholic liver disease remains a leading cause of chronic liver disease, with increased morbidity and mortality. In recent years, biomarkers discovered through metabolomics have shed light on disease biochemistry, arousing interest in ethanol-induced biochemical perturbations. In this study the metabolic profiles of mouse cerebral tissue following short- or long-term ethanol exposure were analyzed. The long-term experiment (8 weeks) consisted of C57BL/6 mice (8-10 weeks old) separated into control and ad libitum feeding with a Lieber-DeCarli ethanol diet group for 8 weeks containing daily 5% alcohol 99%. The short-term animal experiment was conducted over 11 days. In addition, a single dose by oral gavage of 25% alcohol, at the 5th day of the intervention and at the last day of experiment 6 hours before sacrifice were administrated. The experiment was conducted in agreement with current national and European legislation (N. 2015/1992, ΠΔ 56/2013, European guideline 2010/63) and cerebral tissues were collected postmortem. Untargeted metabolic profiling analysis was performed using HRAM Q-TOF-MS (LCMS-9030, Shimadzu) with precursor and product ion scanning at 50 spectra per second within a mass range of m/z 50-1000. Univariate statistical analysis revealed that the short-term experiment decreased arachidonic acid, docosahexaenoic acid, glycerophosphocholine, CDP-choline, adenine, adenosine, cytidine, creatine, creatinine, valine and taurine along with some phospholipids. Long-term exposure decreased acetyl-carnitine, adenine and adenosine with phospholipids and ceramides, while many amino acids were increased along with hypoxanthine, xanthine, oxoproline, choline, creatinine, and docosahexaenoic acid. Metabolomics has proven to be a useful tool in elucidating the toxic effect of alcohol consumption.

P-111 Targeted metabolomics reveals training level specific metabolic adaptations in marathon race athletes

PRESENTING AUTHOR: ***Mark Haid, Helmholtz Zentrum Muenchen, Molecular Endocrinology and Metabolism, Germany***

CO-AUTHORS: ***Jana Schader, Alexander Cecil, Julia Schoenfeld, Martin Halle, Arne Pfeufer, Jerzy Adamski, Johannes Scherr***

Objectives: We were interested in characteristic patterns of metabolism in periods of preparation for and participation in a marathon race of 76 male athletes at different performance levels. Methods: We used a targeted metabolomics approach to analyse blood plasma samples of 76 male subjects who participated in the Munich marathon in 2013 (Enzy-MaGIC-Study). Metabolic changes were analysed at two different time points before and three time points after the marathon race. To reveal characteristic features between runners at different training levels, the cohort was stratified into top, average, and low performers based on general endurance (relative VO2max) and net running time during the marathon race. Results: In general, we observed increased concentrations of acylcarnitines (AC) and reduced concentrations of amino acids (AA) and phospholipids (PL) immediately after the race when compared to pre-exercise levels. In the following 24 hours and 72 hours a massive supercompensation of AA and PL levels became apparent. Furthermore, the metabolite profiles revealed performer class related characteristics in terms of the different periods of structured exercise. Most pronounced differences between performer classes were observed for acylcarnitines and urea cycle related amino acids immediately after the race. In conclusion, our study shows characteristic metabolite patterns between pre- and post-marathon race time points and reveals performance-dependent differences with regards to marathon-induced changes of the metabolome.

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*AWARD WINNERS

BIOMEDICAL

P-112 Differentiation of Fasting and Non-fasting Plasma Samples by Informative Metabolite Sets

PRESENTING AUTHOR: *Patrick Dreher, Helmholtz Center Munich, Germany*

CO-AUTHORS: *Johannes Raffler, Thomas Skurk, Hans Hauner, Robert Mohney, Hannelore Daniel, Karsten Suhre, Gabi Kastenmüller*

Blood metabolites are easily accessible biomarkers that can extend routine clinical chemistry. The utilization of non-fasted blood samples considerably simplifies blood sampling for patients and clinicians. However, the metabolite readout reflects the current metabolic status with substantial differences between fasted and fed states, which need to be considered for clinical interpretation. Additionally, dietary intake has shown to increase subject variability. Therefore, it is vital to know the fasting status at the time of sampling. We propose two machine learning prediction models that provide an objective differentiation of plasma samples into non-fasting (less than 4h or 6h postprandial) and fasting states. We used an iterative random forest approach with backward variable selection, trained on the time-resolved HuMet dataset comprising 56 time points with four different meal and fasting periods in 15 healthy men. In this study, a total of 595 metabolites were identified by non-targeted mass spectrometry-based metabolomics. The 4h prediction model contains nine metabolites that display two distinct mirror-like kinetic patterns: The first group of metabolites including glucose, fructose, and 3-hydroxy-3-methylglutarate, demonstrates elevated metabolite levels during non-fasting and low levels during fasting time points. Six fatty acids (mostly medium-chain) demonstrate a mirror-inverted behavior to the first group. In addition to the same six fatty acids, our 6h prediction model includes nucleotides and bile acids, which show comparatively slower postprandial changes in blood levels. Notably, our models can classify plasma samples with a low error rate (< 8,4%). The usability of our prediction models will be validated in large cohort studies.

P-113 NMR based metabolic analysis of human blood associated with particulate matter in air pollution

PRESENTING AUTHOR: *Seoyoung Jang, Korea Basic Science Institue, South Korea*

CO-AUTHORS: *Juhwan Noh, Changsoo Kim, Geum-Sook Hwang*

Particulate matter (PM) has became a major environmental risk factors all over the world, and related to human disease such as respiratory disease, nervous system disorder. We evaluate the change in response to environmental exposure and to investigate the association between concentrations of identified metabolite in human blood samples and PM with a diameter 2.5-10 μm (PM10). In this study, we analyzed human whole blood from 457 Koreans using 1H-NMR spectroscopy. We performed multivariate linear regression with the level of PM10 adjusted for age, sex, BMI, and smoking status. We found that PM10 was significantly altered energy metabolism such as succinate, pyruvate, lactate, and fumarate. This study demonstrated that NMR based metabolic profiling can be a useful tool and to assess the impacts of environmental pollutants on human metabolism and to identify potential biomarkers for predicting exposure of air pollution like PM10.

P-114 Metabolite profiling of multiple measures of adiposity: A Mendelian randomization analysis

PRESENTING AUTHOR: *Matthew Lee, MRC Integrative Epidemiology Unit, University of Bristol, United Kingdom*

CO-AUTHORS: *Kaitlin Wade, Laura Corbin, Nicholas Timpson*

The mechanisms by which obesity affects disease are poorly understood. Studying different adiposity measures together with intermediate phenotypes reflective of physiological function can provide valuable insight into complicated etiologic disease pathways. We investigated the causal effect of several adiposity measures on metabolic intermediates using Mendelian randomization (MR). This epidemiological approach uses genetic markers as proxy measures for exposures, to avoid typical limitations of observational analyses. A two-sample MR analysis was conducted using genetic variants for three exposures: body mass index (BMI), waist-hip-ratio (WHR), and body fat percentage (BF%). The outcomes comprised 123 metabolites derived from nuclear magnetic resonance spectroscopy. An inverse variance weighted multiplicative random effects model was applied. A newly developed web tool, MR-Vis (http://bristol-medical-stat.bristol.ac.uk:3838/MR-Vis/), was used to visualize and explore causal perturbations of metabolic profiles in relation to exposures. Across all three exposures, the direction of effect was consistent for 106 metabolites. BMI and WHR exhibited similar effects across a range of metabolites, including an inverse association with medium to large HDL and a positive association with a range of LDL and VLDL particles. Many of the results for BF% were consistent with the null. A positive association with glycoprotein acetyls and phenylalanine (P<0.05/123) was observed across all three exposures. The consistency observed in the relationship between different measures of adiposity and metabolic intermediates suggests some redundancy in these measures. However, where we see contrasting associations that can be mapped onto corresponding differences in disease susceptibility, our approach can provide insight into the biological mechanisms underpinning these differences.

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*AWARD WINNERS

BIOMEDICAL

P-115 Joint analysis of metabolite markers of fish intake and persistent organic pollutants in relation to type 2 diabetes risk in Swedish adults

PRESENTING AUTHOR: ***Rikard Landberg, Chalmers University of Technology, Sweden***

CO-AUTHORS: ***Lin Shi, Carl Brunius, Ingvar A. Bergdahl, Ingegerd Johansson, Olov Rolandsson, Carolina Donat Vargas, Hannu Kiviranta, Kati Hanhineva, Agneta Åkesson***

Conflicting evidence exists regarding the association between fish intake and type 2 diabetes (T2D) incidence, potentially due to error-prone self-reported dietary intake assessments and co-exposure to persistent organic pollutants (POPs) present in fish. We identified plasma metabolites associated with fish intake and assessed their association with T2D risk, independently from co-exposure to POPs, in a case-control study nested in the Swedish prospective Västerbotten Intervention Programme cohort. Plasma samples from 421 case-control pairs at baseline and 10-year follow-up samples from a subset of 149 pairs were analyzed using untargeted LC-MS metabolomics. Moreover, 16 plasma POPs were analyzed for the sub-population (149 pairs). We identified 31 metabolites associated with fish intake using multivariate modelling and partial correlation analysis. No association was observed between fish intake either reflected by metabolites or measured by food frequency questionnaires, and T2D risk, using a conventional multivariate logistic regression adjusted for POPs. Interestingly, integrating fish-related metabolites and plasma POPs using two-way orthogonal partial least squares regression led to a unique metabolite component independent of POPs, which was borderline inversely associated with T2D risk (odds ratio:0.75; 95% CI: 0.54, 1.02, P=0.07). This component mainly reflected fatty fish intake and consisted of several species of phosphatidylcholines and phosphatidylethanolamines. Our results support that fatty fish may be beneficial for T2D prevention, although simultaneous co-exposure to POPs may counteract those effects in Swedish adults. Integrating metabolite markers and POP exposures appears a promising approach to advance the understanding of associations between fish intake and T2D incidence in populations co-exposed to POPs.

P-116 Plasma biomarkers of boiled and filtered coffee intake and their association with type 2 diabetes risk

PRESENTING AUTHOR: ***Carl Brunius, Chalmers University of Technology, Sweden***

CO-AUTHORS: ***Lin Shi, Ingegerd Johansson, Ingvar A. Bergdahl, Olov Rolandsson, Kati Hanhineva, Rikard Landberg***

Habitual coffee intake has been associated with a lower risk of developing type 2 diabetes (T2D), but only few studies have investigated different coffee brews, i.e. boiled and filtered, separately. We identified metabolites related to either filtered or boiled coffee, and assessed their association with T2D risk in a nested case-control study within the Swedish prospective cohort Västerbotten Intervention Programme. Plasma samples from 421 case-control pairs at baseline and from a subset of 149 pairs at 10-y follow-up were analyzed using untargeted LC-MS metabolomics. We identified 31 metabolites that were associated with boiled- ($0.1 \leq |r| \leq 0.47$) and 30 associated with filtered coffee intake ($0.1 \leq |r| \leq 0.34$), respectively, using multivariate modelling and partial correlation analysis adjusted for life-style confounders. Among these metabolites, 14 and 10 were unbiasedly selected in panels by LASSO regression for accurate and specific prediction of boiled (AROC =0.91) and filtered coffee (AROC =0.90), respectively. Moreover, metabolite panels could also predict boiled and filtered coffee intake after 10-y of follow-up (AROC=0.71 and 0.80, respectively). Furthermore, we observed an inverse association between the metabolite panel score related to filtered coffee and T2D risk, independent of established risk factors and lifestyle confounders. No association was observed for boiled coffee. In summary, for the first time, we identified metabolite panels which might be used as selective biomarkers for boiled and filtered coffee intake,respectively. Our study supports a protective role of habitual intake of filtered coffee on T2D development.

P-117 Investigating in vivo digestion of sheep and cow milk in a rat model

PRESENTING AUTHOR: ***Linda Samuelsson, AgResearch Ltd, New Zealand***

CO-AUTHORS: ***Linda Samuelsson, Natalie Ahlborn, Wayne Young***

Sheep milk is a rich source of vitamins, minerals and energy, with up to 50% more milk solids than cow milk. Anecdotally, sheep milk is thought to be better tolerated than cow milk and some of these affects have been attributed to differences in milk protein digestibility. However, controlled scientific studies examining this potential difference are lacking. The purpose of this study was to compare the digestion of sheep and cow milk in terms of a) apparent ileal amino acid digestibility and b) metabolite profiles in serum and caecal contents in a rat model. Male Sprague-Dawley rats were fed a dairy-free rodent chow and provided with sheep or cow milk over a 28-day period. Serum, distal ileal content and caecal content were collected. Amino acid digestibility in the distal ileum at day 28 was higher in rats fed sheep milk; amino acid intake in sheep milk rats was 1.8 times higher, but the amino acid concentration in the ileal content was only 1.3 times higher. Concentrations of essential amino acids in serum were significantly higher in rats fed sheep milk, indicating that sheep milk proteins are more readily digested. In addition, rats fed sheep milk had higher concentrations of dimethyl sulfone, g-aminobutyric acid, amino acids and short-chain fatty acids in serum and caecal contents compared to rats fed cow milk indicating that ingestion of the two milks lead to different digestion products. The potential for these products to have differential effects on physiology and health is yet to be established.

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BIOMEDICAL

P-118 Lifestyle-intervention-induced reduction of abdominal fat is reflected by increased serum HDL diameter and decreased glycerol level

PRESENTING AUTHOR: ***Marian Beekman, Leiden University Medical Center, Netherlands***

CO-AUTHORS: ***Marian Beekman, Bianca A.M. Schutte, Erik B. van den Akker, Raymond Noordam, Petra Dibbets-Schneider, Lioe-Fee de Geus-Oei, Joris Deelen, Ondine van de Rest, Diana van Heemst, Edith J.M. Feskens, P. Eline Slagboom***

Abdominal obesity is one of the main modifiable risk factors of age-related cardiometabolic disease. Cardiometabolic disease risk and its associated abdominal fat mass, cholesterol and glucose levels can be reduced by a healthier lifestyle. Hence, our aim is to understand the relation between the lifestyle intervention induced changes in body composition, and specifically abdominal fat, and the accompanying molecular changes. Therefore, we used the data from the Growing Old Together (GOTO) study, in which 164 older adults (mean age 63 years, BMI 23-35 kg/m2) changed their lifestyle during 13 weeks by 12.5% caloric restriction plus 12.5% increase in energy expenditure. Here, we first show that the levels of circulating metabolic biomarkers specifically reflect the abdominal fat mass, even after adjustment for body mass index. Second, we show that the applied lifestyle intervention mainly results in reduction of abdominal fat (-2.6%, SD=3.0) and that this reduction, when adjusted for weight loss, is highly associated with an increase of the HDL diameter and decrease of circulating glycerol levels. To monitor the weight-loss-independent beneficial effects of lifestyle change, circulating glycerol levels and HDL diameter may be valuable tools.

P-119 Metabolic features of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS)

PRESENTING AUTHOR: ***Emi YAMANO, Health Metrics Development Team, RIKEN Compass to Healthy Life Research, Japan***

CO-AUTHORS: ***Masahiro Sugimoto, Satoshi Kume, Yasuyoshi Watanabe, Tomoyoshi Soga, Lucinda Bateman, Suzanne D. Vernon, Yosky Kataoka***

The pathogenesis of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) characterized by exertional and severely debilitating fatigue with/without infectious or neuropsychiatric symptoms lasting at least 6 consecutive months is not fully understood. Because of incomplete understanding of aetiology and diagnostic uncertainty of ME/CFS, there are no firmly established objective diagnosis using biomarkers. In the present study, we performed comprehensive metabolomic analyses of plasma samples obtained from ME/CFS patients and healthy controls in the United States using capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS). By reference to our previous findings of inactivity in tricarboxylic acid (TCA) and urea cycles in ME/CFS (Yamano E, 2016), we focused on the alterations of metabolite concentrations in these metabolic cycles. In ME/CFS patients, malate was increased and arginine was decreased relative to healthy controls. The female patients with ME/CFS notably exhibited abnormalities in TCA and urea cycles, compared with male patients. In addition, arginine/malate ratio discriminated female patients with ME/CFS from female healthy controls yielding area under the receiver operating characteristic curve values of 0.72 (95 % confidential interval: 0.61-0.72, $P < 0.0001$). This suggested that ME/CFS patients in the US may also have some dysregulation of TCA and urea cycles which reflects the pathophysiological state in line with our finding in Japanese samples, and that sex may be the significant factor in the development of the biomarker for ME/CFS. These findings will help to establish the objective diagnosis marker and treatment recommendations for ME/CFS.

P-120 Machine learning of human plasma lipidomes for obesity estimation in a large population cohort

PRESENTING AUTHOR: ***Christian Klose, Lipotype GmbH, Germany***

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Shotgun lipidomics of human plasma provides hundreds of individual lipid measurements in a fast, reproducible, single assay, making it a useful quantitative method for biomarker identification. In this study we trained machine intelligence models to predict obesity estimates, i.e. body mass index (BMI), waist-hip ratio (WHR) and body fat percentage (BFP), on a lipidomic dataset of 1061 samples of the FINRISK 2012 cohort and confirmed the outcome by an independent validation dataset of the Malmö Diet and Cancer Cardiovascular Cohort. Comparison of the different models revealed that the lipidome predicted BFP the best. Moreover, the plasma lipidome also contains information about body fat distribution, since WHR was predicted more accurately than BMI. These modelling results required full resolution of the lipidome to lipid species level. The power of the lipidomics association was demonstrated by the finding that the addition of routine clinical laboratory variables, e.g. HDL- or LDL- cholesterol did not improve the model further. Correlation analyses of the individual lipid species, controlled for age and separated by sex, demonstrated the multi-parametric nature of the correlation with the BFP. Correlation profiles were similar between the three obesity estimates, but very different from those lipids correlating with HDL-, LDL-cholesterol, and triglyceride levels.

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*AWARD WINNERS

BIOMEDICAL

P-121 Metabolite Trajectories Following Contrasting Prudent and Western Diets from Food Provisions: Identifying Biomarkers of Short-term Changes in Habitual Diet

PRESENTING AUTHOR: ***Philip Britz-McKibbin, McMaster University, Canada***

CO-AUTHORS: ***Nadine Wellington, Meera Shanmuganathan, Russell J. de Souza, Sandi Azab, Mateen Sheikh, Michael Zulyniak, Jonathon Bloomfield, Alicia Mell, Ritchie Ly, Dipika Desai, Sonia S. Anand***

Recent changes to habitual diet play important roles in population health, including alarming rises in obesity prevalence and chronic disease risk worldwide. However, few objective biomarkers of recent food intake are used in nutritional epidemiology, which rely on food frequency questionnaires or dietary records prone to bias and selective reporting. Herein, metabolomic analyses were performed on healthy participants (n=42) from the Diet and Genetic Intervention (DIGEST) study based on a parallel two-arm randomized clinical trial. Matching urine and plasma specimens were collected at baseline and following a 2-week assigned Prudent or Western diet, where participants were provided all food items with a weight-maintaining menu plan from a dietician. Targeted and non-targeted metabolite profiling was conducted using three complementary instrumental platforms with stringent quality control to avoid false discoveries. Metabolic phenotype changes were characterized based on distinctive trajectories following 2 weeks of food provisions as compared to baseline habitual diet of participants when using univariate and multivariate statistical models. Unknown metabolites associated with contrasting dietary patterns were identified with high resolution MS/MS and co-elution after spiking with authentic standards. Top-ranked metabolite trajectories were also correlated to self-reported changes in average intake of macro/micronutrients from diet records. For the first time, we have identified robust biomarkers sensitive to short-term changes in dietary patterns from food provisions that are applicable to a healthy and free-living population outside of a laboratory setting.

P-122 A step-wise nutrimetabolomics approach reveals food-specific compounds in urine of adults consuming a DASH-style diet

PRESENTING AUTHOR: ***Nichole Reisdorph, University of Colorado, United States***

CO-AUTHORS: ***Nichole A. Reisdorph, Audrey E. Hendricks, Minghua Tang, Katrina A. Doenges, Richard M. Reisdorph, Brian C. Tooker, Kevin Quinn, Sarah J. Borengasser, Yasmeen Nkrumah-Elie, Daniel N. Frank, Wayne W. Campbell, Nancy F. Krebs***

Although health benefits of the Dietary Approaches to Stop Hypertension (DASH) diet are established, it is not understood which specific compounds in foods are responsible for these benefits. We utilized a step-wise approach to identify unique compounds from individual foods of a DASH-style diet, determined if these food-specific compounds (FSCs) are detectable in urine, and then examined relationships between these urinary-FSCs and blood pressure (BP). Nineteen subjects were randomized into 6-week controlled DASH-style diet interventions. Untargeted, LC/MS-based metabolomics was performed on 24-hour urine samples collected before and after each intervention and on 12 representative DASH-style foods. Linear mixed effects models were used to correlate FSCs to BP changes. Mass spectrometry signals for FSCs were summed to derive a food-specific signature to evaluate nutrikinetics. Between 66-969 compounds were determined to be FSCs and between 13-190 of these FSCs were detected in urine. Nutrikinetics demonstrated a relationship between food consumption and presence of food specific signatures in urine. Although no urinary FSCs were associated with BP, 16 and 6 non-FSC, urinary compounds were associated with BP and changes in BP over time, respectively. These comprised both endogenous and exogenous compounds, including some food-related compounds that were not analyzed by LC/MS/MS in this study. This study establishes a technical, analytical, and conceptual framework upon which future nutrimetabolomics studies can be built. A novel aspect of this study is the use of food-specific signatures to evaluate nutrikinetics.

P-123 Syringol metabolites as biomarkers of smoked meat intake

PRESENTING AUTHOR: ***Roland Wedekind, International Agency for Research on Cancer, France***

CO-AUTHORS: ***Pekka Keski-Rahkonen, Nivonirina Robinot , Vivian Viallon, Pietro Ferrari, Erwan Engel, Marc J. Gunter, Inge Huybrechts, Augustin Scalbert***

Background: Processed meat is associated with higher risk of colorectal cancer but the estimation of intake of this heterogeneous food group in epidemiological studies is challenging because of the lack of sufficient details in dietary questionnaires. Objective: To identify novel biomarkers of intake of processed meat products using metabolomics. Design: An untargeted metabolomic approach based on LC-MS was applied to processed meat products previously digested in vitro, and to urine and plasma samples from a randomized cross-over dietary intervention in which 12 volunteers consumed successively 3 processed meat products and two other control foods during 3 days. The identified biomarkers were then measured in urine from 474 subjects from the European Prospective Investigation into Cancer and nutrition (EPIC) cross-sectional study for which a 24h dietary recall and food frequency questionnaires were available. Results: Syringol and four derivatives of syringol were found to be characteristic of digests of smoked meat products. The same compounds present as sulfate esters in urine showed increased levels following consumption of smoked meat products in the intervention study. They were also positively associated with recent and habitual consumption of smoked meat products in urine samples from participants of the EPIC cross-sectional study. These markers showed good discriminative ability for smoked meat intake with receiver operator characteristic areas under the curve up to 0.86 and 0.79 for short-term and habitual intake, respectively. Conclusions: The biomarkers of smoked meat intake identified in this study may be used to improve assessment of smoked meat intake in epidemiological studies.

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*AWARD WINNERS

BIOMEDICAL

P-124 Association of the metabolome with health parameters and food intake in a prostate cancer cohort; an untargeted metabolomics study

PRESENTING AUTHOR: ***Johnny R Östman, Swedish University of Agricultural Sciences, Sweden***

CO-AUTHORS: ***Rui C Pinto, Elin Thysell, Timothy MD Ebbels, Göran Hallmans, Ali A Moazzami***

Prostate cancer (PCa) is the most frequently diagnosed cancer and the second most common cause of cancer-related death in men. The associations between PCa risk and pre-diagnostic metabolite levels have been previously investigated, but have yielded inconclusive results. We used a liquid chromatography-high resolution mass spectrometry untargeted metabolomics approach to look for metabolites associated with the risk of future PCa in fasting plasma using a nested case-control study design of 777 pairs of PCa cases and their matched controls (n = 1554) recruited from Northern Sweden Health and Disease Study Cohort (NSHDS). In order to avoid variation we only included cases with a follow-up of over 5 years. Within this cohort an additional sub-cohort of cases with aggressive PCa was identified (n = 169 pairs). A range of health parameters (i.a. body mass index, 2-hour oral glucose tolerance test, food frequency questionnaire answers) was collected for all subjects at baseline. The untargeted metabolomics approach identified approximately 3000 features, which were further condensed into approximately 1200 features using a novel batch matching algorithm. The associations of the metabolomic features with the health parameters were investigated using conditional logistic regression for both the whole dataset and the aggressive cancer sub-cohort. Indications of associations between hitherto unidentified metabolomic features and health parameters could be shown, shedding light on observable metabolic changes that might manifest as future PCa.

P-125 Comparison of Serum Non-Esterified and Total Fatty Acids as Reliable Biomarkers of Maternal Dietary Fat Intake: A Pilot Study in the FAMILY Birth Cohort

PRESENTING AUTHOR: ***Sandi Azab, McMaster University, Canada***

CO-AUTHORS: ***Russell DeSouza, Natalie Campbell, Michael Zulyniak, Sonia Anand, Koon Teo, Philip Britz-Mckibbin***

Fatty acids (FAs) are dietary components that have long been implicated in health and disease. However, FAs analysis encompasses diverse fractions derived from blood, erythrocytes or adipose tissue. To date, serum phospholipid and total lipid fractions are often used in epidemiological and nutrition studies with a notable gap regarding the clinical utility of more convenient lipid pools for dietary biomarkers, such as serum non-esterified FAs (NEFA). In this study, 50 subjects from FAMILY birth cohort were selected based on a devised diet index to constitute a “good diet” and a “poor diet” group. Comprehensive analyses of serum NEFA and total FAs were performed using multisegment injection-non-aqueous-capillary electrophoresis–mass spectrometry (MSI-NACE-MS) to investigate associations of circulating FAs with the diet index score, fish, dairy and fiber servings derived from standardized food-frequency questionnaires (FFQs). Pregnant women within the good diet group had higher EPA and DHA ($q < 0.05$; FDR) in both analyzed fractions. These also showed significant correlations with reported omega-3 FAs servings notably non-esterified EPA ($r = 0.46$; $p < 0.0001$). Additionally, serum odd-chain FAs, such as non-esterified C15:0 demonstrated the highest correlation to full-fat dairy intake ($r = 0.43$; $p < 0.001$). For the first time, we demonstrate that direct analysis of serum NEFA using MSI-NACE-MS offers a high-throughput approach for reliable assessment of habitual fat intake that avoids complicated sample fractionation, hydrolysis, derivatization and long analysis times. These findings endorse the role of metabolomics for high-quality observational studies investigating the impact of maternal nutrition on birth outcomes.

P-126 Untargeted metabolomics to reveal red versus white meat-associated gut metabolites in a prudent and Western dietary context

PRESENTING AUTHOR: ***Sophie Goethals, Ghent University, Laboratory of Chemical Analysis, Merelbeke, Belgium, Belgium***

CO-AUTHORS: ***Sophie Goethals, Caroline Rombouts, Lieselot Y. Hemeryck, Lieven Van Meulebroek, Thomas Van Hecke, Els Vossen, John Van Camp, Stefaan De Smet, Lynn Vanhaecke***

The underlying mechanisms of the association between red and processed meat intake and a higher risk for a range of chronic diseases are not fully elucidated yet and can probably be affected by other dietary components. Untargeted metabolomics in the gut is a promising approach to investigate the response of host and microbiome on the consumption of meat in the context of dietary pattern. The impact of red and processed meat versus chicken combined with a prudent or Western dietary pattern on the gut metabolome was investigated by using a four week pig feeding trial as a model for humans. Thirty-two piglets received one of the four dietary treatments mimicking human diets. Untargeted ultra-high performance liquid chromatography coupled to high resolution mass spectrometry (UHPLC-HR-Q-Orbitrap-MS) (Vanden Bussche et al., 2015, Anal. Chem.), preprocessing with Compound DiscovererTM and multivariate statistics using Simca 14.1 and RStudio were applied to characterize the metabolic fingerprints of the pig small intestine and colon digests. The validated OPLS-DA models (R2X>0.35, R2Y>0.99, Q2>0.71 and p-values CV-ANOVA<0.005) investigating the effect of ‘dietary pattern’ and ‘meat type’ could demonstrate a higher impact of dietary pattern compared to meat type on the gut metabolome. The abundance of most meat-associated metabolites did not depend on the dietary pattern. However, some metabolites such as long-chain acylcarnitines were only present at high levels in the pigs fed the ‘Western-Red&Processed’ diet. In addition to the confirmation of previously described chicken and red meat-associated biomarkers (anserine, carnosine, carnitine,...), new interesting metabolites were tentatively identified.

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*AWARD WINNERS

BIOMEDICAL

P-127 The Age Metabolome: A Consortium of Metabolomics Studies (COMETS) Meta-analysis of 91,965 Adults

PRESENTING AUTHOR: ***Steven Moore, NCI, United States***

CO-AUTHORS: ***Steven C. Moore, Ella Temprosa, Ewy Mathe, Krista Zanetti, Jessica Lasky-Su, Rachel S. Kelly***

Age is the most important risk factor for human morbidity and mortality, but its specific metabolic effects are incompletely understood. To clarify the age-metabolism relationship, we evaluated correlations of age with levels of serum and plasma metabolites in 36 cohort studies and 91,965 adults in COMETS. Cohorts were from North America (N=15), Europe (N=13), and Asia (N=6) and the median age at blood collection was 55y (range:18-90y). Metabolomics data were generated predominantly by Metabolon (N=12 cohorts), the Broad Institute (N=7), Nightingale Health (N=7), or from Biocrates kits (N=6). We analyzed cohort-specific Spearman age-metabolite correlations, adjusted for sex, smoking, fasted status, BMI, race/ethnicity, and more. We meta-analyzed results for metabolites measured in at least 6 studies (random-effects). Overall, 1,136 metabolites were measured in at least 6 studies. Across these 1,136 metabolites, the median number of participants with measured values was 13,457, and the maximum was 91,965. In total, 290 metabolites were significantly correlated with age at the Bonferroni significance threshold ($p<0.00004$); 247 correlations were positive, 43 were inverse. The top 10 positive correlations were for neurotransmitter precursors and products, like vanillylmandelic acid (r=0.31;p=2.1*10-27) and C-glycosyltryptophan (r=0.29;p=2.1*10-13), and urea cycle metabolites like 2,3-dihydroxy-5-methylthio-4-pentenoate (r=0.29;p=2.1*10-13) and symmetric dimethylarginine (r=0.23;p=1.8*10-8). The top 10 inverse correlations were sex steroid hormones, e.g. dehydroepiandrosterone sulfate (r=-0.29;p=3.3*10-16). Associations replicated across cohorts, regions, platforms, sex, and BMI and smoking history groups. These findings highlight specific perturbations in metabolism of the central nervous system, the urea cycle, and steroid hormones are associated with older age. Findings were reproducible and generalizable.

P-129 Perfluoroalkyl substances, the plasma metabolome and risk of Type 2 Diabetes - a prospective nested case-control study in a Swedish population

PRESENTING AUTHOR: ***Tessa Schillemans, Karolinska Institutet, Sweden***

CO-AUTHORS: ***T. Schillemans, S. Lin, C. Donat-Vargas, A. Tornevi, M. Wennberg, J. Sommar, I. Johansson, J. Koponen, H. Kiviranta, K. Hanhineva, O. Rolandsson, I.A. Bergdahl, R. Landberg, A. Åkesson, C. Brunius***

Perfluoroalkyl substances (PFAS) are widespread and persistent environmental pollutants. There is evidence that PFAS induce metabolic perturbations, but underlying mechanisms are still unknown. We used untargeted LCMS metabolomics (n=24758 features) to find metabolites related to PFAS exposure in a case-control study on type 2 diabetes (T2D) (n=187 matched pairs) nested within the Västerbotten Intervention Programme cohort. Exposures of six measurable PFAS, were grouped into two patterns by principal component analysis: 1) perfluorononanoic acid, perfluorodecanoic acid and perfluoroundecanoic acid and 2) perfluorooctane sulfonic acid, perfluorooctanoic acid and perfluorohexane sulfonic acid. Using a random forest algorithm, we discovered metabolite features associated with individual PFAS or PFAS exposure patterns, which were subsequently investigated for associations with risk of T2D. PFAS exposures correlated with 154 features ($0.15 \leq |r| \leq 0.35$) after adjustment for gender, age, sample year and case-control status. Two PFAS-related features also associated with T2D after adjustment for matching factors (gender, age, sample year) and for fish and meat intake, but not for BMI. These metabolites, putatively identified as a phosphatidylcholine and a diacylglycerol, had opposite directions of associations with PFAS exposures and T2D risk: The phosphatidylcholine correlated positively with perfluorodecanoic acid (r=0.22) and associated inversely with risk for T2D (OR=0.35; 99.9% CI=0.16-0.79), the diacylglycerol correlated negatively with perfluorodecanoic acid (r=-0.18) and associated directly with risk for T2D (OR=1.93; 99.9% CI=1.02-3.65). Our results suggest that especially perfluorodecanoic acid-related metabolites may have inverse associations with T2D risk.

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BIOMEDICAL

P-130 Untargeted metabolomics profiling of longitudinal urine samples to determine the personalized baseline for integrated personalized omics profiling (iPOP) project

PRESENTING AUTHOR: ***Songjie Chen, Stanford University, United States***

CO-AUTHORS: ***Yuqin Dai, Liang Liang, Michael Snyder***

iPOP project aims at defining personalized health baseline by multi-omics profiling of diverse longitudinal samples collected from individual participants. The easy accessibility and noninvasive sampling of urine make it promising for longitudinal metabolomics analysis. However, such analyses remain challenging due to complexity and broad dynamic range of metabolites. We developed a robust high performance HILIC Q-TOF LC/MS method to profile urine samples with broad coverage of metabolite classes, analytical selectivity and reproducibility despite high sample salt concentration. The method was validated by consistent measurement of a pooled quality control urine sample and further applied to profile samples of an iPOPer with a high sampling frequency. The untargeted metabolomics analysis of a pooled quality control sample found more than 3700 metabolic features. The preliminary results showed reproducible retention time and signal response for 500 sample injections over a one-week of LC/MS data acquisition time. A panel of highly sensitive hydrophilic metabolites such as sugar phosphates and nucleotides were detected with an enhanced signal response by incorporating medronic acid additive.This is the first case in iPOP project to discover the longitudinal personalized urine phenotype by consistent metabolomics profiling.

P-131 Targeted urine metabolomics using UPLC-QTOF-MS combined with a reporting tool is a high throughput screening for IEM

PRESENTING AUTHOR: ***Irene Körver-Keularts, Clinical Genetics, MUMC+, Netherlands***

CO-AUTHORS: ***Steinbusch L K M, Wang P, Waterval W A H, Stassen F, Bierau J, Habets D D J***

BACKGROUND: In an effort to consolidate several dedicated biochemical urine tests and shorten our turn-around-time we developed an innovative targeted urine metabolomics screening containing 226 biomarkers covering 242 OMIM phenotypes and a reporting tool to facilitate diagnosis. METHODS: Creatinine normalized urine samples were spiked with three internal standards (IS) (13C6-galactitol, D4-sebacic acid and D3-hexanoylglycine) and run on a UPLC-QTOF-MS in positive and negative modes. Acquired untargeted data were extracted for the relative signal over IS of targeted analytes in both 10 and 50 ppm mass extraction windows. Peak overloading was automatically corrected and peak shifting and peak quality were notified. Z-score of each analyte was calculated against an age-matched control cohort of 280 samples. Disease phenotypes were based on scores from biomarker matching. Needle plots visualised all abnormal analytes and a heatmap showing the predicted disease phenotypes. RESULTS: 298 unique samples from 273 patients covering 75 OMIM phenotypes were analyzed in our clinical validation. We were able to correctly diagnose, amongst others, organic acidurias and defects in amino acids, purines and pyrimidines and vitamin and cofactors metabolism. Some IEM, eg. sterol and peroxisomal metabolism, need further evaluation in targeted plasma metabolomics. This innovative method reduced the turn-around-time significantly. DISCUSSION: In conclusion, we developed a fast targeted urine metabolomics tool which replaces a number of dedicated urine analyses for efficient screening of patients suspected of an IEM.

P-132 Designing a new class of covalent QS inhibitors to target P. aeruginosa virulence

PRESENTING AUTHOR: ***Shaked Uzi, Student, Israel***

Bacteria have the ability to communicate with each other and sense their population densities through secretion of small signaling molecules called autoinducers, in a process that is known as ‘quorum sensing’ (QS). These days, the increasing prevalence of antibiotic resistance constitutes a worldwide problem and targeting bacteria through QS inhibition may provide a sophisticated new strategy to engage this issue. P. aeruginosa is an opportunistic pathogen which regulates its pathogenicity through QS. Its main autoinducer, 3-oxo-C12-homoserine lactone (C12), binds to the LasR transcriptional regulator protein and activates a cascade of events that regulates biofilm formation and secretion of virulence factors. By targeting covalently the Cys79 residue inside the LasR binding pocket, irreversible inhibition of the QS in P. aeruginosa can be achieved. In this project, a covalent inhibitors containing Michael acceptors with different chain lengths were designed and their bio-activity was examined. Two of the designed probes were found to bind covalently to LasR but surprisingly none of them were able to reduce pyocyanin production, a hallmark of Pseudomonas virulence. On the other hand, even though some of these probes were partial agonists, they still were able to reduce the bacterial virulence and damage its ability to form biofilms.

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BIOMEDICAL

P-133 Towards state-of-the-art in TB Meningitis metabolomics diagnostics

PRESENTING AUTHOR: ***Shayne Mason, Centre for Human Metabolomics, North-West University, South Africa***

The infectious agent (M. tuberculosis) responsible for TB can spread to the brain; inflammation of the meninges leads to TB meningitis (TBM). Diagnosis requires clinical, chemical, microbiological and PCR analysis of cerebrospinal fluid (CSF) — the diagnostic gold standard, but unfortunately sometimes inconclusive. A missed/delayed diagnosis staggers correct treatment, leading to severe neurological morbidities, or even death, especially in paediatrics. Through metabolomics, we developed a defining biochemical profile of neuroinflammation that, in time, will become a differential diagnostic point-of-care method for TBM. Based on untargeted 1H-NMR metabolomics on CSF, we proposed the astrocyte-microglia lactate shuttle for TBM, which gained increased recognition following our experimental support through LC-MS and amino acid profiling results. We're currently exploring CSF metabolomics of TBM and other forms of meningitis, aimed at validating a differential CSF diagnostic profile for TBM. Urinary GC-MS metabolomics holds promise for a non-invasive method of diagnosis and prognosis for TBM, complemented with our studies and across other types of infectious diseases (e.g. HIV), which alert to commonalities within the gut-liver-brain axis. Advanced metabolomics technology (GCxGC-TOF-MS) will serve to further characterize neuroinflammatory markers in the urine. Finally, we believe CSF metabolomics using a new ionic profiling method assessing changes of ion-channels and transporters within the brain will open a state-of-the-art diagnostic approach to neuroinflammation. With our established metabolomics expertise (50% of outputs on TBM metabolomics), combined with other omics data, a defined biochemical profile of neuroinflammation seems to be in the offering — an enterprise for which international collaboration would be indispensable.

P-134 The Immunomodulatory Effect of Propolis In THP-1 Derived Macrophages and in Primary Macrophages

PRESENTING AUTHOR: ***David Watson, University of Strathclyde, United Kingdom***

CO-AUTHORS: ***Abdulmalik Alqarni, Samyah Alanazi, Naif D. Alenzi, Samya Alenezi, James Fearnley, Hugo Fearnley, Valerie A. Ferro, William Harnett***

Propolis is collected by bees from the plants in the environment around their hives and its composition varies according to the available plants. In temperate regions it is largely collected from poplar buds. It is used to coat hive surfaces as an anti-infective being particularly active against protozoal species. It is a complex mixture and it has multiple biological effects. In the current study propolis extracts from different geographic origins were assessed for their anti-inflammatory activities by investigating their ability to alter the production of tumour necrosis factor-α (TNF-α) and cytokines in THP-1 derived macrophage cells (THPMs) co-stimulated with lipopolysaccharide (LPS) and in primary macrophages stimulated with LPS. In the THPMs the propolis extracts suppressed the levels of TNF-α and IL-6 cytokines produced by LPS-stimulation. Similar suppression effects were detected for IL-1β, but the release of this cytokine was synergised by propolis samples from Ghana and Indonesia when compared with LPS. Overall, a Cameroonian propolis extract (P-C) was the most immunosuppressive and this was evaluated for its effects on the metabolic profile of unstimulated macrophages or macrophages activated by LPS. Metabolites were identified by liquid chromatography coupled with mass spectrometry. LPS altered energy, amino acid and nucleotide metabolism in THP-1 cells. A very specific effect of the P-C propolis appeared to be the inhibition of purine nucleotide phosphorylase. Extracts of propolis from Brazil and the UK were found to inhibit the immune response in primary murine macrophages and in this case the target appeared to be nitric oxide production.

P-135 Investigating Canine Hepatocutaneous Syndrome Through an Untargeted Metabolomic Approach

PRESENTING AUTHOR: ***M. Elena Diaz-Rubio, Cornell University, United States***

CO-AUTHORS: ***Adam J. Miller, John P. Loftus, Luis P. Macho, Sharon A. Center, Sheng Zhang***

Hepatocutaneous Syndrome (HCS) is a canine disease characterized by a distinct hepatopathy and cutaneous lesions in conjunction with hypoaminoacidemia. Aminoaciduria has been recently identified as component of this syndrome and lysinuria specifically has been identified as a consistent feature of this syndrome. Many HCS associated metabolic abnormalities may relate to altered amino acid metabolism. However, comprehensive links between changes in amino acid concentrations other metabolic alterations remain unresolved. Therefore, an untargeted metabolomics approach analyzed plasma from dogs with HCS and compared them to healthy dogs of similar ages and breeds to further characterize this disease. Plasma from dogs with HCS (n=19) and healthy control dogs (n=9) were analyzed. Chromatographic separation was performed using Hilic method on a Vanquish UHPLC coupled to a QE-HF mass spectrometer. All samples along with the QCs were run in negative and positive ion modes and the acquired data were processed using Compound Discoverer 3.0. Metabolites were identified with Compound Discoverer 3.0 software through the mzCloud HRAM fragmentation library. Statistical Analyses were performed with Metaboanalyst 4.0 and SIMCAP + software (Umetrics, Sweden). Preliminary results indentified significant differences ($P < 0.01$) between HCS and healthy dogs in a variety of metabolites. These metabolites are involved in pathways that include vitamin B6 metabolism, the citric acid cycle, pyrimidine metabolism, pantothenate and CoA synthesis, and various amino acid metabolic systems. These results begin an in-depth analysis of metabolic perturbations that define HCS. We have identified metabolites as candidate biomarkers and novel treatments target for HCS.

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BIOMEDICAL

P-136 Metabolomic Investigation of Immune Cell Activation

PRESENTING AUTHOR: ***Florence A. Castelli, CEA : French Alternative Energies and Atomic Energy Commission, France***

CO-AUTHORS: ***Amir Louarr, Aurélie Delvaux, Patricia Lamourette, Emeline Chu-Van, Evelyne Correia, Bernard Maillere, Christophe Junot, François Fenaille, Florence Anne Castelli***

In recent years, it has been shown that some specific metabolites could be involved in immune cell polarization during infection, thus shaping the immune response. Within the adaptive immune system, dendritic cells (DCs) play a central role, orienting the immune response according to the perceived danger signal. While metabolism seems to significantly impact DC activation, the underlying mechanisms are still poorly described and understood. We aimed to develop a comprehensive method to get deeper insight into DC metabolic reprogramming. Monocyte-derived dendritic cells were In vitro activated either with lipopolysaccharide (LPS, bacterial signal) or poly:IC (viral signal) and their corresponding metabolic responses studied by an untargeted LC/HRMS-based metabolomics approach. Statistical data analyses were used for thoroughly assessing specific metabolic differences. We described the first comprehensive metabolic map of DCs (> 150 metabolites robustly monitored) and identified dramatic metabolic changes upon DC activation with LPS (>100 metabolites impacted), whereas bacterial and viral DC activations can be distinguished by significant fluctuations in more than 70 metabolites. Those Metabolomic analyses revealed alterations in four main metabolic pathways (alanine-aspartate-glutamine, glutathione, and pyrimidine pathways, Krebs cycle), significantly upregulated upon bacterial infection and downregulated in the case of viral infection. The few metabolites already described were confirmed while many others were also highlighted, thus enabling a better comprehension of the metabolic mechanisms of pathogen-specific DC activation. Our approach represents an unprecedented, powerful and straightforward way to robustly monitor metabolic activation of immune cells that would facilitate the discovery of biomarkers specifically signing bacterial or viral infection.

P-137 Discovering metabolic biomarkers for predicting efficacy of anti-histamine treatment in chronic urticaria patients

PRESENTING AUTHOR: ***SiHyun Chae, Seoul National University, School of Medicine, South Korea***

CO-AUTHORS: ***Da Jung Kim, Andrew HyoungJin Kim, Min-Gyu Kang, Joo-Youn Cho***

Chronic urticaria is a chronic inflammatory skin disease that occurs for more than six weeks and recurs frequently. First line treatment of chronic urticaria is anti-histamine, which is proven to be effective but bares large inter-individual variation in response. Thus, the aim of this study was to discover metabolic biomarkers for predicting therapeutic outcome of anti-histamine treatment in patients with chronic urticaria by using metabolomics approach. Total 60 plasma samples were collected from patients suffering from chronic urticaria. Twenty five of them showed response to anti-histamine and thirty five of them were diagnosed as non-responders. Plasma metabolomes were analyzed using GC-TOF-MS. Multivariate statistical analysis were conducted where biomarkers were selected according to fold change difference between two groups with false discovery rate adjusted P value less than 0.05. As a result, palmitic acid and stearic acid were selected as putative biomarkers, which were significantly higher in non-responder group. Therefore, we speculate changes in lipid metabolism are linked with the efficacy of anti-histamine treatment in chronic urticaria and by understanding the mechanism, the therapeutic outcome of anti-histamine treatment in chronic urticaria patients can be predicted.

P-138 Metabolomics as a tool to discover metabolite biomarkers for cow milk allergy diagnosis

PRESENTING AUTHOR: ***Lynn Vanhaecke, Ghent University, Belgium***

CO-AUTHORS: ***Ellen De Paepe, Lieven Van Meulebroek, Eric Cox***

In industrialized nations, food allergies are a growing epidemic and are considered a major thread to our wellbeing. Cow milk allergy is one of the first allergies to occur in early childhood and early life sensitization has been associated with an increased risk to develop the atopic march, including eczema, asthma and other food allergies later in life. As such, more research is urgently needed to gain more insights into this disease. Therefore, this study evaluated a unique multi-matrix platform for polar metabolic fingerprinting of feces, plasma and urine, applying UHPLC-Q-OrbitrapTM-HRMS, to determine the optimal matrix for future research on cow milk allergy in children. Plasma is popular for metabolomic analysis, but collection is problematic in young children, while feces and urine are readily available biofluids. All three fingerprinting approaches were proven 'fit-for-purpose' through extensive validation in which excellent linearity (R2 > 0.99 or 0.90 respectively), recovery and precision (coefficient of variance < 15% or 30% respectively) were observed. The effectivity of the platform was demonstrated by subjecting simultaneously collected fecal, urine and plasma samples from 10 healthy volunteers to metabolic profiling and fingerprinting, yielding respectively 9672, 9647, and 6122 components, with a substantial overlap of the plasma metabolome with fecal (69.48%) and urinary samples (76.79%). Orthogonal partial least-squares discriminant analysis provided similar results for feces and plasma in gender-discrimination (p-value = 0.036), suggesting feces as a promising alternative biofluid to plasma for food allergy research. The latter was confirmed by highly promising results in preliminary experiments.

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BIOMEDICAL

P-139 Metabolic perturbations in response to enteropathogen infections in children from resource constrained settings

PRESENTING AUTHOR: ***Natasa Giallourou, Integrative Systems Medicine and Digestive Diseases, Department of Surgery and Cancer, United Kingdom***

CO-AUTHORS: ***Fahmina Fardus-Reid, Gordana Panic, Eric Houpt, Margaret Kosek, Jonathan Swann***

Early-life enteropathogen infections are major drivers of diarrhoea and stunted growth in children living in under-resourced settings. Infections and malnutrition continue to impact the growth and development of millions of children worldwide, despite a variety of anti-microbial, nutritional and hygiene interventions as well as improved access to vaccination programs. Untargeted 1H NMR spectroscopy was used to characterize the metabolic profile of urine samples collected longitudinally from children from Peru (n=273 infants, N=1057 samples), Tanzania (n=249 infants, N=506 samples) and Bangladesh (n=249 infants, N=860 samples) in the first two years of life, participating in the multisite birth cohort study (MAL-ED). We estimated associations between metabolomic data and data obtained from quantitative PCR used to detect 29 enteropathogens in stools collected from the same cohorts. Shifts in gut microbial metabolites arising from the catabolism of amino acids like tyrosine, tryptophan and phenylalanine were associated with pathogens like Giardia, Norovirus, Campylobacter and Escherichia coli pathotypes. Additionally, energy expenditure appears to be modified in infected children reflected in shifts of nicotinamide metabolism. Urinary lactose levels were positively associated with viral infections suggesting increased intestinal permeability in infected children. The majority of infection-related metabolic shifts were also observed in children with poor growth outcomes. This work has generated new insights into the biochemical perturbations of enteric infections in infants from developing countries, providing a metabolic framework from which nutritional programs and interventions for environmental enteric dysfunction can be more precisely constructed and evaluated.

P-141 Superbug Surveillance

PRESENTING AUTHOR: ***Soren Wacker, University of Calgary, Canada***

CO-AUTHORS: ***Ian Lewis, Sergei Y. Noskov, Annegret Ulke-Lemee, Thomas Rydzak, Yonatan Grad, Deirdre Church, Fiona Clement, Ashlee Earl, Christopher Naugler, Rauf Salamzade***

The rise of antibiotic-resistant bacteria is a problem so serious that it threatens modern medicine around the globe. Half of the common bacteria are already resistant to front-line drugs. New extremely resistant microbes are already emerging which cannot be cured with conventional drugs anymore. Calgary Laboratory Services (CLS) has collected bacteria from the population of Calgary (~1 million people) within the past 20 years, including every single strain cultured from bloodstream infections. At the University of Calgary, these bacteria are re-grown and dismantled with a multi-omics approach (metabolomics, proteomics, genomics) to understand the spread and the origins of multi-drug resistance, in the worldwide largest population-based study addressing infectious diseases.

P-142 The mode of action of T. gondii tissue cyst inhibitors

PRESENTING AUTHOR: ***Deborah Maus, Robert Koch-Institute, Junior Group 2, Berlin, Germany, Germany***

CO-AUTHORS: ***Jens Pikkemaat, Elyzana Dewi Putrianti, Martin Blume***

The intracellular, apicomplexan parasite Toxoplasma gondii infects up to 30% of the global human population and causes life-threatening diseases in immuno-compromised patients. Chronically persisting bradyzoites form cysts in brain and muscle tissues and are responsible for transmission and remission of this disease. However, currently available medical treatment options are only effective against the virulent tachyzoites but fail to target the chronic stages of T. gondii. To address this shortcoming, we are screening antimicrobial compound libraries against both stages of the parasite in a fluorescence-based assay in a plate format. To identify the molecular target of inhibitory compounds we pursue an untargeted metabolomics approach using HILIC-UHPLC-MS: The metabolic fingerprint of treated parasites will be compared with the impact of characterized inhibitors with known modes of action. In subsequent reverse-genetic, knock-down und fluxomics experiments we will further characterize the potential T. gondii cyst inhibitor and its molecular target.

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***AWARD WINNERS**

BIOMEDICAL

P-143 Taking the metabolomic approach to look at multiple organ dysfunction syndrome (MODS) in trauma patients

PRESENTING AUTHOR: ***Anna Karen Laserna, National University of Singapore, Singapore***

CO-AUTHORS: ***Jorming Goh, Lynette Loo, Jialing Neo, Pavandip Singh Wasan, Mikael Hartmann, Mahesh Uttamchandani, Philip Iau, Sam Fong Yau Li, Shabbir Moochhala***

The developments in trauma care have led to a decrease in immediate and early mortality among trauma patients. However, with the decrease in early trauma mortality, a new cause of death in the intensive or post-operative care units was found, wherein patients succumb to complications in organs unrelated to the initial injury. It is called multiple organ dysfunction syndrome (MODS), which was defined as a condition of altered organ function in which an acutely ill patient cannot be maintained in homeostasis unless provided with medical intervention. This arises as a consequence of an overwhelming systemic inflammatory response. In this pilot study, we take a metabolomic approach to assess the development of MODS in relation to the metabolic perturbations of patients through time and try to identify the association of metabolites with organ-specific or multiple organ dysfunction. The study was done with 23 trauma patients admitted to the surgical intensive care and high dependency units having different degrees of injury severity at admission. Plasma samples were collected within the first 12 hours of inclusion in the study and every 12 hours thereafter until the 48th hour. Patient condition was evaluated using the sequential organ failure assessment (SOFA) scoring system. Untargeted metabolomic analyses were performed using reverse phase and hydrophilic interaction LC-MS. Multivariate analysis revealed that the metabolomic profile can be used to establish a total SOFA score threshold to distinguish MODS and non-MODS patients. Certain metabolites were also found to have significant correlation with MODS and organ-specific dysfunction.

P-144 Effects of PHMG, a humidifier disinfectant, on eye-dryness using LC-MS-based metabolite profiling

PRESENTING AUTHOR: ***Hyang Yeon Kim, Dankook University, South Korea***

CO-AUTHORS: ***Jung Dae Lee, Jueng Eun Im, Soo bean Oh, Hyeyoon Goo, Kyong Jin Cho, Kyu-Bong Kim***

Eye-dryness is multifactorial disease on eye and caused by many reasons such as contaminated external environment and use of systemic medications. In Korea, people who used humidifier disinfectant contained polyhexamethylene guanidine (PHMG) appealed discomfort in their eye. To investigate if PHMG worsens eye-dryness syndrome, we instilled PHMG solution (0.1% or 1%) to eyeball of rat, which was induced eye-dryness by scopolamine (3mg/kg for 7days). In this study, we used benzalkonium chloride (BAC), which is known as inducer of eye-dryness of positive control (PC). After application of PHMG for 5 days, the changes of tear production, break up tears film (BUT), corneal damage, number of lacrimal gland neutrophil and conjunctiva goblet cell were observed. The tear production, corneal damage, number of lacrimal gland neutrophil and detached cornea epithelium cells were increased in eye-dryness group compared with control group, while BUT and number of conjunctiva goblet cell were decreased. In addition, IL-6, IL-1β and TNF-α in cornea and gland were also significantly changed. Almost immune markers were increased in eye-dryness groups compared with control group. Analysis of PHMG effect on eye-dryness using liquid chromatography and mass spectrometry (LC-MS) were performed on plasma samples using SIMCA software. In partial least squares-discriminant analysis (PLS-DA), the clustering of control was clearly separated from eye-dryness, PC and PHMG groups. Moreover, eye-dryness group was differed from PHMG and PC groups. Some organic acid and lysoPCs and 10 non-identified compounds were selected as biomarkers of eye-dryness and deterioration of the symptom based on variable important plot (> 1.0).

P-145 Investigation of Plasmodium falciparum metabolome by UHPLC-ESI-HRMS: case study of two chemoresistant strains

PRESENTING AUTHOR: ***Denis Desoubzdanne, French Armed Forces Biomedical Research Institute (IRBA), France***

CO-AUTHORS: ***Laura Desnouveaux, Jérôme Dormoi, Nicolas Taudon***

Malaria is still one of the most widespread parasitic tropical diseases. Moreover, parasitic chemoresistance towards different antimalarial drugs is still expanding, whereas the biochemical mechanisms of its agent are so far poorly understood. In this context, we have proposed to investigate intra-parasitic metabolome of two Plasmodium falciparum strains by UHPLC-ESI-HRMS approach. The parasites were synchronized in schizonte stage after a Sorbitol treatment and a magnetic field separation (VarioMACS™ Separator, Miltenyi Biotec). In one hand, the African Mefloquine resistant strain 3D7 was exposed to the active drug Chloroquine at three doses (0x, 0.5x and 1xIC50 for 3 h. In the other hand, the Asiatic Chloroquine resistant strain W9 was exposed to the active drug Mefloquine at two doses (0x and 1xIC50) for 3 h. Parasites were isolated to the red blood cells with a Saponine treatment and washed with PBS. Intra-parasitic metabolites were extracted by microwaves in cold MeOH. After phospholipids + proteins depletion and evaporation to dryness, metabolites were re-suspended in acidified water/MeOH. They were separated with a Kinetex® Biphenyl LC column (Phenomenex) and then analyzed in both ESI+ and ESI- modes with an UHPLC-ESI-HRMS instrument (1290-6550A QTOF, Agilent). Metabolomics data were processing and normalized thanks to the open-source web-based W4M platform developed by the French metabolomics community (Giacomoni F. et al., 2014). Multivariate biostatistical analyses (PLS-DA, ANOVA) were realized in order to highlight putative signals correlated to the strain and/or the dose effects.

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BIOMEDICAL

P-146 Integrative analysis of in vivo host-parasite Metabolome, Genome and Transcriptome in malarial African children

PRESENTING AUTHOR: ***Wael Abdrabou, New York University, United Arab Emirates***

CO-AUTHORS: ***Mame Massar Dieng, Aïssatou Diawara, Aboubacar S. Coulibaly, Amidou Diarra, Vinu Manikandan, Alfred B. Tiono, Sodiomon Sirima, Issiaka Soulama and Youssef Idaghdour***

Plasmodium falciparum (Pf) malaria stands as one the most serious threats to global public health placing almost half of the world population at risk of infection. Symptoms of Pf malaria appear during the intra-erythrocytic developmental stage of the parasite when infected Red Blood Cells rupture releasing more parasites into the blood. However, host response to the infection can vary dramatically between individuals and between different ethnic groups through poorly understood mechanisms. During blood stage, P. falciparum engages with host in metabolites exchange and triggers de novo biogenesis pathways to ensure steady supply of nutrients needed for parasite proliferation. In this research project, we conduct a longitudinal matched in vivo study that aims at describing metabolic perturbations in sub-Saharan African children during Pf malaria and investigating if and how metabolites could modulate host response to infection. Our results reveal distinct metabolic processes and biochemical signatures in the serum samples of the infected children compared to their metabolomic profiles prior to infection. The overall goal of the study is to investigate metabolic profiles, and associated genotypic variants and gene expression traits using an integrative multi-omics approach. Achieving this goal will advance our understanding of the sources of variation in the host-parasite interactions taking place during malaria infection and provide a more holistic and systemic view to the biochemical-gene networks associated with the infection.

P-147 Metabolic Response to Supplemental Carnitine Identifies a Distinct Septic Shock Phenotype of Early Mortality

PRESENTING AUTHOR: ***Kathleen Stringer, University of Michigan College of Pharmacy, United States***

CO-AUTHORS: ***Michael Puskarich, Charles Evans, Alla Karnovsky, Alan Jones***

Background: Metabolic phenotyping of sepsis is challenged by patient heterogeneity. To better understand the metabolic derangements associated with early (28d) sepsis mortality, we are using metabolomics data from septic shock patients treated with supplemental L-carnitine. Methods: Temporal serum metabolomics data from non-survivors and survivors enrolled in the carnitine dose-ranging phase of a clinical trial (NCT01665092) were compared. P values were corrected for false discovery (FDR). Results: At T0, non-survivors (n=9), had higher median levels of measured acylcarnitines and a higher median C2:carnitine ratio (FDR<10%) versus survivors (n=12). Non-survivors also had T0 elevations in proline, histidine, and oleic acid (18:n-9) (FDR<15%). Carnitine (6, 12, 18 g) supplementation resulted in a non-dose dependent increase in median peak (T24) carnitine concentration (IQR) which was higher in non-survivors (562, 350-1132μM) versus survivors (222, 159-512μM; FDR=16%). Acyl-carnitines and C2:carnitine ratio remained elevated in non-survivors. Post-carnitine levels of proline and histidine declined in non-survivors but oleic acid remained elevated and stearic acid (18:0) progressively increased (FDR=16%). Demographic and clinical data (age, total SOFA, lactate and BMI) were not different between groups. Median (IQR) T0 renal SOFA was higher in non-survivors (1.0 [0-1] v. 2.0 [1-2.5], p=0.04) but there was no association between it and peak carnitine. Conclusion: Carnitine-treated non-survivors have evidence of disrupted carnitine metabolism prior to treatment that may contribute to elevated carnitine and acyl-carnitine levels in response to carnitine supplementation. These patients also have an inflammatory metabolic profile that may preclude their therapeutic responsiveness to carnitine and contribute to their mortality.

P-148 Elevated plasma and urine biomarkers for active Onchocerca volvulus infections

PRESENTING AUTHOR: ***Rob Vreeken, Janssen R&D, Belgium***

CO-AUTHORS: ***Ole Lagatie, Emmanuel Njumbe Ediage, Dirk Van Roosbroeck, Stijn van Asten, Ann verheyen, Bieke Van Dorst, Linda Batsa Debrah, Alex Debrah, Ruben T'Kindt, Koen Sandra, Lieve Dillen, Filip Cuyckens and Lieven Stuyver1***

The neglected tropical disease Oncocherciasis, or River Blindness, is caused by an infection with the filarial nematode Onchocerca volvulus. Approximately 17 million people, majority living in Africa, are infected world-wide. Until today there is neither a cure or a non-invasive diagnostic test available. Next, to enable PKPD studies with novel microfilaricide drug candidates, surrogate end-points and efficacy biomarkers are in high demand but non-existent. Here we report on the use of multimodal untargeted mass-spectrometry based biomarker discovery to identify onchocerciasis-associated metabolites and lipids in urine and plasma (O.volvulus infected cases: 68 individuals with palpable nodules; lymphatic filariasis cases: 8 individuals; non-endemic controls: 20 individuals) . We identified 2 plasma metabolites, viz. inosine and hypoxanthine linked to filarial infection and one urine metabolite, i.e. cis-cinnamoylglycine, related to O. volvulus infection. Markers were confirmed (sensitivity, specificity) during a separate targeted validation experiment, using UPLC-HR-MS and UPLC-HR-MS/MS analysis. Next steps will focus on the application of these markers in microfilaricide clinical trials, MDA efficacy surveys and epidemiological transmission studies.

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BIOMEDICAL

P-149 Viral and bacterial infection elicit distinct changes in plasma lipids in febrile children

PRESENTING AUTHOR: ***Xinzhu Wang, Imperial College London, United Kingdom***

CO-AUTHORS: ***Ruud Nijman, Stephane Camuzeaux, Caroline Sands, Marieke Emonts, Jethro Herberg, Ian Maconochie, Matthew Lewis, Michael Levin, Myra McClure***

Fever is the most common reason that children present to Emergency Departments (EDs) in the UK. Clinical signs and symptoms suggestive of bacterial infection are often non-specific, and no test exists for the accurate diagnosis of infection. As a result, many children with viral infection are prescribed antibiotics unnecessarily, while others with serious bacterial infections are not treated in a timely manner and progress to sepsis. In recent years, the 'omics' approaches to identifying biomarkers from the host-response to bacterial infection are proving promising. In this study, lipidomic analysis was carried out with plasma samples obtained from febrile children with confirmed bacterial infection (n=20) and confirmed viral infection (n=20). We show for the first time that bacterial and viral infection elicit distinct changes in the host lipidome. Glycerophosphoinositol, sphingomyelin, lysophosphotidylcholine and cholesterol sulate were increased in the confirmed virus infected group, while fatty acids, glycerophosphocholine, glycerophosphoserine, lactosylceramide and bilirubin were increased in cases of confirmed bacterial infection. A combination of 20 metabolites increased diagnostic performance and achieved the AUC value of 0.853 (95% CI, 0.672 - 0.995). This pilot study demonstrates the potential of metabolic biomarkers to distinguish bacterial from viral infection in febrile children, to facilitate effective clinical management and to limit inappropriate use of antibiotics.

P-150 The circulating metabolome in the progression to islet autoimmunity and type 1 diabetes

PRESENTING AUTHOR: ***Santosh Lamichhane, Turku Centre for Biotechnology, University of Turku, Finland***

CO-AUTHORS: ***Santosh Lamichhane, Linda Ahonen, Thomas Sparholt Dyrlund, Esko Kemppainen, Heli Siljander, Heikki Hyöty, Jorma Ilonen, Jorma Toppari, Riitta Veijola, Tuulia Hyötyläinen, Mikael Knip, Matej Oresic***

Type 1 diabetes (T1D) is a chronic autoimmune disease caused by specific destruction of the insulin-producing beta cell. Clinically pre-diabetic period in T1D is characterized by presence of beta cell specific autoantibodies. Intriguingly, earlier metabolomics study suggests specific metabolic disturbances before individuals precede to auto-immunity. Here we combine lipidomics and metabolomics approach to analyze circulating metabolites in a prospective series of plasma samples from 40 children who progressed to T1D (PT1D), 40 children who developed at least a single islet autoantibody but did not progress to T1D during the follow-up (P1Ab) and 40 matched controls (CTR). We found sphingomyelins and methionine to be persistently dysregulated in PT1D when compared to the P1Ab and CTR groups. Additionally, phosphatidylcholines and triacylglycerols were downregulated in PT1D as compared to P1Ab, while amino acids including glutamine, aspartic acid were downregulated in PT1D as compared to CTR at the age of 3 months. Furthermore, we found hydroxyphenyllactic acid, indole acetic acid, and 11-eicosenoic acid, metabolites of potential microbial origin, to be significantly downregulated at early age (3 and 6 months) preceding clinical T1D. Our study support findings from earlier studies and suggests novel metabolic signatures that specifically characterize children who progressed to islet autoimmunity or overt T1D, respectively, which may be helpful in the identification of at-risk children before the initiation of autoimmunity.

P-151 Baseline metabolic profiles of early rheumatoid arthritis patients achieving sustained drug-free remission after initiating treat-to-target TCZ,MTX or the combination:insights from systems biology

PRESENTING AUTHOR: ***Wei Yang, Leiden University, Netherlands***

CO-AUTHORS: ***Xavier Teitsma, Amy C Harms, Alireza Mashaghi, Thomas Hankemeier***

For patients with newly diagnosed rheumatoid arthritis (RA), treatment aim is early, rapid, and sustained remission. However, still a significant number of patients respond insufficiently to first-line drug methotrexate (MTX) or new biological drug tocilizumab (TCZ). Metabolic analysis prior to therapy could be potential indicators of disease activity and predictors of treatment response. The aim of this study was to identify relevant metabolites and biological pathways associated with achieving sustained drug-free remission (sDFR) after a treat-to-target TCZ- or MTX-based strategy. Baseline serum samples were analyzed from sDFR and non-sDFR patients of early RA. Metabolomic measurements were performed on oxidative stress, amine and oxylipin platforms followed by pathway analyses in each arm. In a result, baseline metabolic profiles of early RA were found to be different between sDFR and non-sDFR and associated pathways can indicate treatment-specific connections with drug mechanisms of MTX or TCZ. Integrated with our previous observations analyzing relevant transcripts and proteins within the same patients, network analysis showed good correlation across "omics" data. Therefore, signature metabolite biomarkers identified in early RA could potentially act as key prognostic factors for applying personalized care in the future.

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BIOMEDICAL

P-152 Analysis of Smoker's and Non-Smoker's Urine using enhanced two-dimensional chromatographic resolution and high performance TOFMS

PRESENTING AUTHOR: ***Nick Jones, LECO Europe BV, Netherlands***

Urine is a favored biological fluid for medical testing, since it is easy to obtain in large quantities and provides a window into an individual's exposure, diet, and general health. In this study, a novel analytical approach based on comprehensive two-dimensional gas chromatography-high performance time-of-flight mass spectrometry was utilized for robust identification of compounds in two urine standard reference materials (smoker's and non-smoker's urine). After sample preparation and derivatization, the urine samples were analyzed using both GC-TOFMS and GCxGC-TOFMS. An increase of confidently identified compounds in urine standards was the direct result of transitioning from GC-TOFMS to high performance GCxGC-TOFMS, which yields cleaner spectra and thus improved spectral similarity scores.The Pegasus BT 4D facilitated fast and confident compound identification through enhanced two-dimensional chromatographic resolution and high performance TOFMS. GCxGC-TOFMS contour plots were highly structured, showing clustered classes of compounds and provided high quality spectral data, that were searched against large, well-established databases. Library hits were filtered using retention index software tools and findings further supported by calculating mass delta values for molecular and fragment ions. Comprehensive data was processed via non-targeted and targeted methods. Comparison of smoker's and non-smoker's results demonstrated increased quantities of tobacco related compounds, such as cotinine and trans-3'-hydroxycotinine, but also phenols, and additional nitrogen-containing compounds.

P-153 Enhanced Quantification of LPA 18:1 in Plasma with SelexION™ Ion Mobility Technology

PRESENTING AUTHOR: ***Cyrus Papan, Sciex Germany GmbH, Germany***

Lysophosphatidic acid (LPA) is a signaling molecule in the class of lipid mediators, functioning by signaling through G-Protein coupled receptors or nuclear receptor proteins. LPA has been associated with a wide range of biological processes. The major bottleneck for accurate quantitation of LPA in plasma is the chemical interferences, using current analytical methods. The most abundant LPA fragments, the best fragments for quantification has strong matrix interferences, while the specific qualifier fragment, the fatty acid, shows very low signal intensity. Here we utilize SelexION Technology for the quantification of LPA 18:1 in plasma which removes the unspecific matrix interferences in plasma sample, allowing to use the abundant MRM transitions for quantitation of LPA with much greater confidence.

P-154 Metabolomics of congenital growth hormone deficiency (GHD) patients treated with recombinant human growth hormone (rhGH)

PRESENTING AUTHOR: ***Nilson Assunção, Unifesp, Brazil***

CO-AUTHORS: ***Luciani Renata Silveira Carvalho, Emanuel Carrilho, Vinicius Guimarães Ferreria, Paulo Cesar Gonçalves Pereira, Breno San Martin***

Diagnosis of GHD is commonly associated with the increase on the occurrence of cardiovascular disease in the patients, that frequently suffers from fatigue, reduced aerobic exercise capacity, abdominal obesity and reduced lean body mass. A variety of these features could be reversed by the use of recombinant human growth hormone (rhGH) replacement, however, the use of rhGH for long periods was previously reported to increase the risks of the patients to suffer cardiac problems. This work aims to better understand the effects of rhGH replacement treatment in the metabolism of the patients, in order to brighten the use of such treatment. Therein, we performed untargeted metabolomics on 46 GHD patients and 12 health controls serum samples. The metabolites were extracted from the serum samples through a protein precipitation protocol, and followed to direct infusion analysis on a high-resolution mass spectrometer (LTQ-Velos Orbitrap, Thermo-scientific). The resulting spectras were first aligned, using the m/z's, by an in-house build software, due the lack of commercial softwares able to handle with direct infusion data. The aligned spectras were then submitted to PCA and PLS-DA analysis using the MetaboAnalyst 4.0 tool. The multivariate analysis indicated a list of higher expressed metababolites in the GDH group, such as glycerol, trimethylamine, citrate, pyruvate, carnitine, betaine, glucose, glycine, tyrosine, leucine, choline. The accuracy of ROC Curve was satisfactory (AUC=0.974). Our preliminary result showed that the most expressive metabolites are related to the process of Lipolysis and the use of lipid reserves in bioenergetic processes.

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*AWARD WINNERS

BIOMEDICAL

P-155 From Molecular Profiling to Precision Medicine in Metabolic Syndrome

PRESENTING AUTHOR: ***Estelle Pujos-Guillot, Clermont Auvergne University, INRA, UNH, Plateforme d'Exploration du Métabolisme, MetaboHUB Clermont, France***

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Metabolic syndrome (MetS), defined as a cluster of cardio-metabolic factors including obesity, hypertension, dysglycemia, and dyslipidemia, and mostly affecting older adults, is now a public health challenge because of its growing prevalence. In the context of personalized medicine/nutrition, new tools are necessary to bring additional knowledge about MetS etiology, better stratify populations and customise strategies for prevention. The objective of this study was to investigate the integration of data from complementary untargeted metabolomics platforms (HRMS, RMN) and technologies to characterize the MetS phenotypic spectrum. A case-control study was designed within the Quebec NuAge cohort1. Six complementary untargeted metabolomic/lipidomic approaches, available within the MetaboHUB infrastructure2, were performed on serum samples collected at recruitment and 3 years later. Standard operating procedures were designed to guaranty the inter-laboratory standardisation from sample preparation to data processing. Data analyses were performed using reproducible online Galaxy workflows3. A full feature selection strategy was developed to build a comprehensive molecular MetS signature, stable over time. Consistent cross-sectional and longitudinal data were observed with a wide range of metabolites (lipids, carbohydrates, amino-acids, peptides...) reflecting subject stability regarding MetS, and providing new insights about underlying mechanisms. Correlation network analysis contributed to explore the links between the molecular signature and clinical parameters. Additionally, an optimized reduced signature was proposed, allowing good prediction performances (12% misclassification, AUC=0.96, CI:[0.94-0.98]), for future clinical application. These results demonstrated the interest of a multidimensional molecular phenotyping as part of the next generation of medicine tools in the frame of non-communicable diseases.

P-156 Faecal metabolomics by conventional UHPLC-HRMS as well as novel LA-REIMS reveals relevant metabolic perturbations in type 2 diabetes

PRESENTING AUTHOR: ***Lieven Van Meulebroek, Ghent University, Belgium***

CO-AUTHORS: ***Cameron Simon, Lapauw Bruno, Takats Zoltan, Vanhaecke Lynn***

Currently, treatments of type 2 diabetes hold some essential shortcomings, which concurs with the fact that this disease is still not curable nor a steady-state can be achieved. In addition, diagnostic tests have limited sensitivity and specificity, and are not ideal for large-scale screening. A better understanding of the underlying mechanisms of type 2 diabetes is therefore desired. For this purpose, our study implemented faecal metabolomics whereby faecal samples were collected from individuals with normal glycaemia (n=24) and type 2 diabetes (n=24) (UZ Ghent EC/2016/0673, HbA1c-based classification). Analytical methodologies employed conventional UHPLC-Q-ExactiveTM-MS as well as innovative Q-ToF-based LA-REIMS, with the latter having potential for point-of-care disease management and large-scale diagnostic screening. Based on the faecal UHPLC-HRMS fingerprints (6620 polar and 19831 lipophilic components), PCA-X score plots revealed clustering according to health state, which was confirmed by OPLS-DA modelling (p-values ≤ 1.86e-9 and Q2Y ≥ 0.654). Tentative identification of the discriminating metabolites revealed some interesting chemical classes, for which a role in type 2 diabetes was substantiated by literature. LA-REIMS was successfully developed and applied for faecal analysis, thereby using a FELS-25A IntelliguideTM CO2 laser. A major asset of the LA-REIMS approach concerned the short total acquisition time per sample, being < 0.5 min. Using this high-throughput approach, discrimination according to health status was possible, as indicated by the valid OPLS-DA model (p-value of 6.65e-17 and Q2Y of 0.767). In conclusion, for the first time, faecal metabolomics was successfully applied to characterize type 2 diabetes, using conventional UHPLC-HRMS and novel LA-REIMS.

P-157 Lipidomic signatures of non-alcoholic fatty liver disease

PRESENTING AUTHOR: ***Aidan McGlinchey, Örebro University, Sweden***

CO-AUTHORS: ***Cecilia Carlsson, Quentin Anstee, Tuulia Hyötyläinen, Matej Orešič***

Nonalcoholic fatty liver disease (NAFLD), a major risk factor for chronic liver disease and type 2 diabetes, has no non-invasive diagnostic techniques currently available for stages such as steatosis, nonalcoholic steatohepatitis (NASH) and fibrosis. We previously identified specific serum lipid signatures associating (1) with total liver fat1 and (2) NASH2. Here, we investigated lipidomic profiles of the European project Elucidating Pathways of Steatohepatitis (EPoS) cohort (n=688), comprising various stages of NAFLD (n=666), NASH (n=661) and fibrosis (n=511). In line with previous studies1, we show that steatosis grade strongly associates with (1) certain low-carbon-number, low-double-bond-count triglycerides increasing and (2) specific phospholipids decreasing. As NAFLD progresses from an earlier steatosis state to a later, severe fibrotic stage, fibrosis grades are used as clinical markers of progression to and severity of NASH. Preliminary analysis of 511 subjects with grades of fibrosis versus those without, revealed distinct relationships existing between circulating lipids and fibrosis stage, the profile changing appreciably between steatosis and fibrosis. We therefore suggest dysregulation of lipid metabolism across stages of NAFLD are reflected in circulation and may hold diagnostic value and insight into NAFLD pathogenesis. Further analysis of these markers is warranted and currently being undertaken taking these preliminary findings further toward diagnostic utility.

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BIOMEDICAL

P-158 Identification of metabolite biomarkers of incident chronic kidney disease in Pre- and Type 2 Diabetes patients

PRESENTING AUTHOR: *Jialing Huang, Helmholtz Zentrum München, Deutsches Forschungszentrum für Gesundheit und Umwelt (GmbH), Germany*

CO-AUTHORS: *Cornelia Huth, Martina Troll, Barbara Thorand, Christian Gieger, Jerzy Adamski, Annette Peters, Rui Wang-Sattler*

Background and objective. Hyperglycemia exerts detrimental effects on the kidney and is one of the leading causes of chronic kidney disease (CKD). Identification of candidate metabolite biomarkers of CKD in hyperglycemic patients and evaluated their involvement in the pathogenesis of CKD are the aims of this study. Methods and Results. We used targeted metabolomics approach and examined longitudinal association of baseline concentrations of 125 metabolites with CKD incidence over 7 years in the KORA (Cooperation Health Research in the Region of Augsburg) cohort. We found that 19 metabolites nominally associated ($P < 0.05$) with incident CKD in 397 hyperglycemic participants in two multivariate logistic regression models by adjusting the effect of known CKD risk factors (e.g. age, systolic blood pressure, fasting glucose, serum triglycerides, baseline estimated glomerular filtration rate and baseline albumin-to-creatinine ratio). Furthermore, three metabolites were selected as candidate biomarkers via Priority-Lasso and a backward stepwise selection, and they were also FDR significant among 125 metabolites in both models. Higher levels of these three metabolites were found to increase the risk of incident CKD in hyperglycemia. Moreover, these three metabolites significantly improved the predictive value of the clinical predictors as the AUC (area under the receiver operating characteristic curve) value increased from 0.854 to 0.887($P = 0.037$). Conclusion. We identified three novel metabolites to be predictors for CKD incidence in hyperglycemia, which may help develop new strategies to prevent CKD in hyperglycemic patients. Our results need to be replicated in an independent study.

P-159 Plasma phospholipid profile may reflect beneficial effect of physical activity on psychological stress

PRESENTING AUTHOR: *Stefania Noerman, University of Eastern Finland, Finland*

CO-AUTHORS: *Elina Järvelä-Reijonen, Anton Mattsson, Urho Kujala, Sampsa Puttonen, Riitta Korpela, Marjukka Kolehmainen, Kati Hanhineva*

Psychological stress seems to increase risk factors of metabolic diseases, but the underlying mechanisms have not been completely understood. Here we present the application of non-targeted metabolite profiling to reveal a panel of metabolites that may potentially intermediates of cross-talk between psychological and physiological health. Subjects prone to metabolic syndrome and psychological distress were recruited from three different cities in Finland and randomised to different 8-week lifestyle interventions. Metabolic profiling was performed on plasma samples of 64 controls and 60 subjects from face-to-face intervention group who showed the largest improvement of stress level between baseline (week 0) and follow-up (week 36). Non-targeted metabolite profiling was performed with high-performance liquid chromatography coupled with tandem mass spectrometry analysis. Physical activity seems to be associated with recovery and negatively associated with stress and adiposity. Several phospholipids that were elevated in the intervention group, including phosphatidylcholine (PC) (18:1/22:6), and two unknown PC (817.596@12.58 and 839.603@12.72) were associated with recovery and peak oxygen consumption (VO2max) and negatively associated with adiposity, stress index, and inflammation marker interleukin-1-receptor antagonist. Conversely, some other PC that were suppressed in intervention group including PC (16:0/16:1), (16:1/18:2), and (18:0/20:3), correlated positively with adiposity and negatively with sleep, recovery, and VO2max. The beneficial effect of physical activity on improvement of stress and body composition hence may be related to changes in the plasma profile of phospholipid, especially ones from PC class. Trial registration: The study was registered at ClinicalTrials.gov on August 17, 2012 with identifier NCT01738256.

P-160 The PXR-gut microbiota cross talk controls the host hepatic lipid metabolism in a sexually dimorphic way

PRESENTING AUTHOR: *Sharon Barretto, INRA-Toxalim, France*

CO-AUTHORS: *Frederic Lasserre, Lorraine Smith, Celine Lucowikz, Arnaud Polizzi, Colette Bétoulières, Laurence Guzylack, Sandrine Menard, Elodie Person, Sandrine Bruel, Daniel Zalko, Cecile Canlet, Lindsay Peyriga, Edern Cahoreau, Laila Mselli-Lakhal, Nicolas Loiseau, Laurence Gamet-Payrastre, Hervé Guillou, Sandrine Ellero-Simatos*

The pregnane X receptor (PXR) is a xenobiotic nuclear receptor, mainly expressed in liver and intestine. In germ-free mice, hepatic PXR activity is significantly reduced, suggesting PXR activation by gut microbiota-derived metabolites. However, its interaction with the gut microbiota remains unclear. We investigated this bi-directional interaction by looking into the effects of gut microbiota suppression by antibiotics integrating transcriptomics (liver, ileum), 1H-NMR based metabolomics (liver, caecal content), lipidomics (liver) and 16S sequencing (caecal content) in Pxr+/+ and Pxr-/- male and female C57Bl6/J littermates. Microbiota suppression significantly decreased Pxr activity in the liver and ileum of males while only in the liver of females. Antibiotics significantly affected the hepatic 1H-NMR aqueous metabolic profiles of both Pxr+/+ and Pxr-/- mice. Antibiotics also decreased hepatic triglycerides, relative abundance of hepatic C16 and C16:1n-7 fatty acids and increased C20:1n-9 fatty acid profile of Pxr+/+ males compared to its control group. Similarly, mRNA levels of elongases (Elovl2, 3 and 5) were decreased in antibiotic-treated Pxr+/+ males only. Reciprocally, PXR deletion significantly affected gut microbiota composition, with increased proportion of Tenericutes in caecal content of Pxr-/- compared to Pxr+/+ males. Metabolic profiles of caecal content were also significantly different, with a tyrosine-derived gut microbiota metabolite significanlty higher in Pxr-/- compared to Pxr+/+ males. No gut microbiota difference was observed in females. In conclusion, gut microbiota activates Pxr both in liver and in ileum of male mice. This crosstalk induces distinct changes in hepatic lipid composition influencing hepatic metabolism and gut microbiota, specific to males only.

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BIOMEDICAL

P-161 Intergrated analysis of bile acid metabolome and micriobiome for High-fat-diet effects

PRESENTING AUTHOR: ***Huiru Tang, School of Life Sciences, Human Phenome Institute, Fudan University, China***

CO-AUTHORS: ***Hong Lin, Yanpeng An, Yulan Wang***

Obesity has become a worldwide health issue though mechanistic aspects of obesity development remain to be fully understood. Here, we aim to elucidate the roles of bile acids and their associations with gut microbiota during obesity development with a high fat diet (HFD)-induced obesity rat model. We developed an UHPLC-MS method for bile acids quantification and analysed bile acids fluxes in multiple biological matrixes including feces, plasma, liver tissue and various segments of intestinal tissues. We found that, irrespective of dietary regimes, taurine-conjugated bile acids were the dominant species in the liver whereas unconjugated bile acids were in plasma. However, HFD caused slight increases in the total bile acids pool and particularly the increases in the levels of deoxycholic acid (DCA) (138.67 ± 37.225 nmol/L in controls, 242.61 ± 43.16 nmol/L in HFD group, p = 0.014) and taurodeoxycholic acid (TDCA) (2.8 ± 0.247 nmol/g in controls, 4.5 ± 0.386 nmol/g in HFD group, p =0.0018) in plasma and liver tissues, respectively, which were consistent with the increased levels of DCA in intestinal tissues and feces. These changes are correlated to an increase in abundance of genera Blautia, Coprococcus, Intestinimonas, Lactococcus, Roseburia, and Ruminococcus. Our investigation revealed the fluxes of bile acids and their association with gut microbiota during obesity development and explicated unfavorable impact of HFD on health.

P-162 Using multivariate and multi-omics approaches to investigate autism spectrum disorder, asthma and Alzheimer's disease

PRESENTING AUTHOR: ***Beatriz Galindo-Prieto, Inst. for Computational Biomedicine, EIPM, Dept.of Physiology and Biophysics, Weill Cornell Medicine, United States***

CO-AUTHORS: ***Jan Krumsiek***

Asthma, autism spectrum disorder (ASD) and Alzheimer's disease (AD) affect millions of people worldwide. Multivariate and multiblock statistical models are widely used for finding relationships and patterns that can help to better understand the underlying biology, and thus move forward to try to reach a cure. Multi-omics data integration entails analyzing multiple -omics data matrices simultaneously by using multiblock approaches that find correlations among the blocks (i.e., data matrices) and relationships between descriptors (e.g., metabolites) and responses (e.g., clinical outcomes). Three examples are provided here: (i) A study carried out at NTNU (Norway) where is shown how multivariate methods such as principal component regression of eye and hand motion tracking data can help to better understand language processing in children with ASD (Vulchanova et al., manuscript in revision, 2019), (ii) a study from UMU and Karolinska Institute (Sweden) of the biological interactions in asthma using the MB-VIOP variable selection method (Galindo-Prieto, 2017) after modeling a 6-block multi-omics dataset (Reinke et al., 2018), and (iii) a currently ongoing study (Arnold et al., bioRxiv, 2019) from Weill Cornell Medicine (USA) with the goal to find correlations between blood and brain in a 4-block multi-omics Alzheimer's dataset. Acknowledgements: We acknowledge past financial support from IDS (Umeå University) (BGP), MKS Instruments (BGP), and the ERCIM 'Alain Bensoussan' Fellowship Programme (BGP), and current financial support from the National Institute on Aging and Weill Cornell Medicine (JK, BGP). We also want to thank Prof. R. Kaddurah-Daouk and the Alzheimer Disease Metabolomics Consortium for their support.

P-163 New biomarkers of 3-hydroxy-3-methylglutaryl CoA lyase deficiency

PRESENTING AUTHOR: ***Štěpán Kouřil, University Hospital Olomouc, Czech Republic***

CO-AUTHORS: ***Jan Václavík, Lucie Mádrová, Radana Karlíková, David Friedecký, Štěpán Kouřil, Leo AJ Kluijtmans, Ron A Wevers, Tomáš Adam***

3-OH-3-Me-glutaryl-coenzyme A lyase deficiency (HMGCLD) is a rare inherited metabolic disorder caused by mutations in HMGCL gene. The mitochondrial enzyme is responsible for catalysing the cleavage of HMG-CoA to acetyl-CoA and acetoacetic acid. Diagnosis is established by tandem MS based newborn selective metabolic screening that contains SRM transition for C5-OH carnitine (262 → 85) which represents four different compounds (Me-malonylcarnitine, 3-OH-isovalerylcarnitine, succinylcarnitine and 2-Me-3-OH-butyrylcarnitine) pointing to different inborn errors of metabolism (IEM). In order to distinguish between these IEM organic acid profiles of urine samples must be acquired. We run untargeted metabolomic analysis of five plasma samples from HMGCLD patients together with a group of controls with the aim to find new diagnostic biomarkers that would be detectable even via newborn screening. Apart from known biomarkers such as 3-OH-isovalerylcarnitine and 3-Me-glutarylcarnitine, other acylcarnitine (AC) species derived from intermediates in the leucine degradation pathway were observed. Furthermore, organic acid counterparts of these AC were also significantly increased compared to controls. Newly found elevated AC could hypothetically be also expected in one other condition, 3-Me-glutaconyl-CoA hydratase deficiency (MGCA). HMGCLD could be distinguished from MGCA by measuring of 3-OH-3-Me-glutarylcarnitine or 3-OH-3-Me-glutarate. The result suggests that 3-OH-3-Me-glutarylcarnitine and 3-OH-3-Me-glutarate could be specific biomarkers of HMGCLD in dried blood spots by FIA-MS screening and thus speed up differential diagnoses among all the diseases screened by C5-OH transition. That would allow for earlier introduction of dietary intervention directly from first screening sample. The work was supported by the Czech Science Foundation Grant (18-12204S) and NPUI (LO1304).

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*AWARD WINNERS

BIOMEDICAL

P-164 Detail LC-MS/MS analysis of organic acids, acylglycines and acylcarnitines for targeted metabolomic profiling

PRESENTING AUTHOR: *David Friedecký, University Hospital and Palacky University Olomouc, Czech Republic*

CO-AUTHORS: *Jaroslava Jáčová, Kateřina Mičová, Tomáš Adam*

Analysis of organic acids offers an important insight into central metabolism. It has traditionally been performed using routine GC-MS platform that requires lengthy sample preparation and allows analysis of just a small batches of samples. The GC-MS under common conditions is not capable to analyze acylcarnitines (together with organic acids) and acylglycines as polar compounds suffer from poor detection. The LC-MS/MS multiple reaction monitoring (MRM) method was developed on the UltiMate (Dionex) LC triple quadrupole 6500 (Sciex) MS for the analysis of 124 organic acids, acylcarnitines and acylglycines in biological matrices. Under acidic conditions of formic acid on non-polar C18 column, good separation of isomers was obtained. Through MRM transitions, detection and quantification of compounds present in urine, serum and intracellular content were enabled. The run time takes 26 min. Analytes show good linearity ($r2 > 0.99$) and reproducibility (CV < 10%) of results. The method was tested on metabolomic experiment on plasma samples of glutaric aciduria I and maple syrup urine disease, where good segregation of groups was obtained, and the diagnostic markers of the disorders were found as the most discriminating compounds. The method is also usable for diagnostic purpose to find inborn errors of metabolism, where selected diseases were tested (SCADD, methylmalonic, glutaric and isovaleric aciduria). The method that minimizes GC-MS drawbacks can be applied as a sensitive and simple approach for targeted clinical metabolomics. It was approved using samples of patients with inherited metabolic disorders. Moreover, it enables accurate and early diagnosing more than 60 disorders.

P-165 Correlation between the metabolome and mtDNA heteroplasmy in mitochondrial disease

PRESENTING AUTHOR: *Marianne Venter, North-West University, South Africa*

CO-AUTHORS: *Jeremie Zander Lindeque, Kazuto Nakada, Roan Louw*

Primary mitochondrial disease caused by variation in mitochondrial DNA (mtDNA) pose difficulties in diagnostics and treatment, due to extensive heterogeneity in clinical presentation of patients: different mtDNA mutations can lead to similar clinical presentations, while the same mtDNA mutation can present with distinct and unrelated clinical phenotypes. Factors involved in this clinical heterogeneity may include heteroplasmy levels (where two or more species of mtDNA can co-exist in a single mitochondrion/cell/tissue), haplogroup context, or other adaptive mechanisms, but remain largely unclear. Only one mouse model has successfully been engineered to harbour an mtDNA deletion, known as ΔMitomice. This mouse model has a large mtDNA deletion and presents with a Leigh Syndrome-like phenotype at high heteroplasmy levels, making it useful for in vivo investigations of mitochondrial disease. The aim of this study was to give new insight into the role of heteroplasmy levels of defective mtDNA in disease progression and severity, using the ΔMitomice disease model. We employed an untargeted metabolomics approach to investigate the changes in metabolite levels along an mtDNA heteroplasmy gradient (0-80% heteroplasmy in tail snips). While some metabolites correlated in a linear fashion with heteroplasmy levels, other metabolites indicated a sudden increase at 60% heteroplasmy, indicating the influence of a threshold effect on these compounds. To our knowledge, this is the first study to utilise linear statistics to investigate the relationship between mtDNA heteroplasmy levels and the metabolome. The findings identified specific metabolites that can be utilised to monitor disease progression and treatment efficacy in mitochondrial disease patients.

P-166 Workflow and normalization strategies employed on CHRIS epidemiological study for targeted metabolomics

PRESENTING AUTHOR: *Vinicius Veri Hernandes, Researcher, Italy*

CO-AUTHORS: *Johannes Rainer, Andrew Hicks, Peter Pramstaller, Sigurdur Smarason*

The Cooperative Health Research in South Tyrol (CHRIS) is a population-based study which aims to investigate mainly neurological and cardiovascular conditions, as well as the process of ageing. More than 13,000 volunteers have been part of the program, which includes clinical tests (blood glucose, antinuclear antibodies, iron metabolism markers and liver function markers, among others), psychological and life style surveys and blood sampling for genotyping and metabolomics study. Using both targeted and untargeted approaches, the objective is to enhance metabolite coverage, maximizing the information obtained for this cohort. Targeted method is based on the Absolute IDQ p180 Kit from Biocrates, which provides a reproducible workflow for sample preparation and the quantification of around 180 metabolites through a liquid chromatography - mass spectrometry (LC-MS) system. The workflow is optimized according to the LC-MS equipment employed at the analysis step (in this case, a QTRAP 6500 from AB Sciex) and each sample set is prepared in the way that 2 derivative subsets are acquired. First subset is used for reversed phase analysis (covering amino acids and biogenic amines) and the second is submitted to a flow injection analysis (FIA) for accessing hexose, acyl carnitines and glycero and sphingolipids. Here we present the overall workflow and methodology for the targeted metabolomics approach of the CHRIS study and results from the employed normalization strategy and quality assessments.

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*AWARD WINNERS

BIOMEDICAL

P-167 Mutations in PCYT2 disrupt etherlipid biosynthesis and cause a complex hereditary spastic paraplegia

PRESENTING AUTHOR: ***Fred Vaz, Amsterdam UMC, Netherlands***

CO-AUTHORS: ***John H. McDermott, Mariëlle Alders, Saskia B. Wortmann, Stefan Kölker, Mia L. Pras-Raves, Martin A. T. Vervaart, Henk van Lenthe, Angela C. M. Luyf, Hyung L. Elfrink, Kay Metcalfe, Sara Cuvertino, Peter E. Clayton, Rebecca Yarwood, Martin P. Lowe, Simon Lovell, Richard C. Rogers, Antoine H. C. van Kampen, Jos P.N. Ruiter, Ronald J. A. Wanders, Sacha Ferdinandusse, Michel van Weeghel, Marc Engelen, Siddharth Banka***

CTP:phosphoethanolamine cytidylyltransferase (ET), encoded by PCYT2, is the rate-limiting enzyme for phosphatidylethanolamine synthesis via the CDP-ethanolamine pathway. Phosphatidylethanolamine is one of the most abundant membrane lipids and is particularly enriched in the brain. We identified five individuals with biallelic PCYT2 variants clinically characterised by global developmental delay with regression, spastic para- or tetraparesis, epilepsy and progressive cerebral and cerebellar atrophy. Using patient fibroblasts we demonstrated that these variants are hypomorphic, result in significantly decreased ET protein levels and enzyme activity without affecting mRNA levels. The significantly better survival of hypomorphic CRISPR-Cas9 generated pcyt2 zebrafish knockout compared to a complete knockout, in conjunction with previously described data on the pcyt2 mouse model, indicates that complete loss of ET function may be incompatible with life in vertebrates. Lipidomic analysis revealed profound lipidomic abnormalities in patient fibroblasts impacting both neutral etherlipid and etherphospholipid metabolism. Plasma lipidomics studies also identified changes in etherlipids that have the potential to be used as biomarkers for ET deficiency. In conclusion, our data establish PCYT2 as a disease gene for a new complex hereditary spastic paraplegia and confirm that etherlipid homeostasis is important for the development and function of the brain.

P-168 HPLC-MS/MS acylcarnitine profiling gives insight into mitochondrial and peroxisomal processes of amino acid and fatty acid metabolism

PRESENTING AUTHOR: ***Pieter Giesbertz, Department of Nutritional Physiology, Technische Universität München, Germany***

CO-AUTHORS: ***Josef Ecker, Alexander Haag, Jarlei Fiamoncini, Hannelore Daniel***

Acylcarnitines are intermediates of fatty acid and amino acid metabolism derived from the conversion of Acyl-CoA species by the enzymatic action of carnitine acyltransferases. In contrast to CoA species, acylcarnitines are present in plasma and urine. In these body fluids, they are important diagnostic markers for inherited diseases of peroxisomal and mitochondrial oxidation. Although a direct injection tandem mass spectrometry might be suitable as a high-throughput strategy, for instance in neonatal screening, the quantification of isomeric and low-concentrated compounds can become challenging. Here, we describe an HPLC-supported MS/MS method for the comprehensive quantification of 85 acylcarnitine species. The method includes most amino acid-derived compounds, as well as odd-numbered carbon species, like pentadecanoyl- (C15) and heptadecanoylcarnitine (C17). Within the context of the metabolic syndrome, the method was applied to human plasma and murine plasma and tissue samples of obesity, insulin resistance and type 2 diabetes. In samples from both species, significant changes were found for acylcarnitine species derived from branched-chain amino acid metabolism. In addition, significant changes in acylcarnitine species derived from peroxisomal alpha and omega oxidation pathways were observed. In a second step, the rather unexplored pathways of peroxisomal fatty acid metabolism were studied in more detail in murine models of peroxisomal stimulation. In conclusion, the method described here extends the current spectrum of acylcarnitine species as markers of mitochondrial and peroxisomal metabolism. The application to human and murine samples gives novel insight in the changes of mitochondrial and peroxisomal metabolism in conditions of aberrant health.

P-169 Ultra high performance tandem mass spectrometric determination of steroid hormones in adipose tissues of two species

PRESENTING AUTHOR: ***Jutta Lintelmann, Helmholtz Zentrum München, Research Unit Molecular Endocrinology and Metabolism, Germany***

CO-AUTHORS: ***Jutta Lintelmann, Katharina Schuh, Helga Sauerwein, André Tchernof, Janina Tokarz, Cornelia Prehn, Jerzy Adamski***

The reliable quantification of steroid hormones in various tissues is an indispensable tool for the investigation and elucidation of metabolic processes involved in course and development of many diseases. We developed and applied a UHPLC-MS/MS method for the determination of 17 steroid hormones in bovine and human adipose tissue. The method includes homogenization and methanolic extraction of the steroids followed by robotized solid phase extraction on well plates of the AbsoluteIDQ® Stero 17 Kit from Biocrates (Biocrates Life Sciences AG). Resulting extracts are separated on a core-shell column (Kinetex XB-C18, 1.7 µm, 150 x 2.1 mm I.D., Phenomenex) using an acetonitrile-methanolgradient and 0.1 % formic acid at 0.3 mL/min and 45 °C (1290 Infinity II, Agilent). Analytes are detected by using a triple quad mass spectrometer (QTRAP 5500, Sciex) with electrospray ionization in positive and negative MRM mode. Method validation resulted in recoveries between 20 % and 63 % making the use of individual internal standards constraining. Intra- and interday imprecisions were acceptable with coefficients of variation from 3 % to 18 %; and detection limits were between 0.01 ng/g and 0.5 ng/g adipose tissue. To demonstrate the applicability of the method, human and bovine subcutaneous adipose tissues were analyzed. First results showed concentrations for the steroid hormones detected lying between 0.07 ng/g (11-deoxycorticosterone) and 15 ng/g (dehydroepiandrosterone) in human adipose tissue and between 0.04 ng/g (estradiol) and 46 ng/g (progesterone) for bovine adipose tissue. The respective patterns and concentrations were highly different between the two species compared.

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*AWARD WINNERS

BIOMEDICAL

P-170 Salivary metabolomics as a new tool for unravelling obesity pathways in adolescents

PRESENTING AUTHOR: ***Kathleen Wijnant, University of Ghent, Belgium***

CO-AUTHORS: ***Kathleen Wijnant, Lieven Van Meulebroek, Beata Pomain, Stefaan De Henauw, Nathalie Michels, Lynn Vanhaecke***

High prevalence and treatment resistance of obesity urges further exploration in early diagnosis and prevention. This is especially important at young age, when metabolic and psychological development are ongoing. Indeed, genetic susceptibility, bacterial symbiosis, but also environmental factors favor obesity. Researchers struggle with the complex processes towards disease susceptibility. Therefore, this project aims to elucidate pathways involved in obesity and obesity-related comorbidities by using salivary metabolomics, both targeted and untargeted. As saliva can be non-invasively collected while its metabolic composition parallels that of blood, it is a very interesting bio-fluid for both research, diagnosis and prognosis. Following the development of a salivary UHPLC-Q-HRMS profiling and fingerprinting method, its 'fit-for-purposeness' was proven through comprehensive validation (targeted 33 endogenous components, untargeted 8564 components) in which excellent precision (CV <15% or 30% respectively), linearity (R2 >0.99 or 0.90 respectively) and recovery were observed. The potential of this method was demonstrated in a first setup, where 13 obese were compared to 13 healthy children (10-16y). The constructed OPLS-DA model enabled us to discriminate adolescents (obese versus healthy weight), reflected by a promising Q2 of 0.669 and R2 of 0.541, good permutation testing and CV-ANOVA p <0.001. Subsequently, 6 metabolites with differentiating potential were retained with satisfying VIP-score (≥1), jack-knifed confidence interval and S-plot descriptors. Integrative metabolomics data analysis strategies will be applied for the identification of differentiating metabolites, pathway analysis and network mapping. This first model proves the potential of salivary metabolomics as a tool for unravelling mechanistic information and biomarker discovery in obesity.

P-171 Lipid remodelling of adipocyte cell membranes is associated with transcriptional and epigenetic changes during genetic and diet-induced obesity in mice

PRESENTING AUTHOR: ***Julian Griffin, Imperial College London, United Kingdom***

CO-AUTHORS: ***Ke-di Liu, Animesh Acharjee, Lee D. Roberts, Melanie K Gulston, Antonio Murgia, Xinzhu Wang, Yajing Chu, James A. West, Robert C Glen, Andrew J. Murray***

Obesity is a complex disorder where the genome interacts with diet and environmental factors to influence ultimate body mass, composition and shape. Numerous studies have investigated how bulk lipid metabolism of adipose tissue changes with obesity, and in particular how the composition of triglycerides (TGs) change with increased adipocyte expansion. However, reflecting the analytical challenge posed by examining non-TG lipids in extracts dominated by TGs, the glycerophospholipid (PLs) composition of cell membranes has been seldom investigated. We conducted a comprehensive lipidomic study of white adipose tissue in mice who become obese either through genetic modification (ob/ob), diet (high fat diet) or a combination of the two using solid phase extraction and LC-MS/MS to study the lipidome and targeted methods to study metabolites involved in one-carbon metabolism and the methylation of cytosine. Pre-fractionation of lipids using SPE allowed the characterisation of a diverse range of PLs which would normally be a minor component of a total lipid extract from adipose tissue. We demonstrate that the changes in TGs that dominate the overall lipid composition of white adipose tissue are distinct from the compositional changes of PLs, the predominant components of the cell membranes. PLs correlate better with transcriptional and one-carbon metabolism within the cell. To investigate epigenetic changes of the genome we developed a targeted analysis of methyl-cytosine and cytosine from DNA. This demonstrates that both genotype and diet alter global methylation of DNA, suggesting the compositional changes that occur in cell membranes have diverse functional consequences during adipocyte expansion.

P-172 Atlas of Circadian Metabolism Reveals System-wide Coordination and Communication between Clocks

PRESENTING AUTHOR: ***Anna Artati, Helmholtz Zentrum Muenchen, Germany***

CO-AUTHORS: ***Kenneth A. Dyar, Dominik Lutter, Nicholas J. Ceglia, Yu Liu, Danny Armenta, N. Henriette Uhlenhaut, Pierre Baldi, Jerzy Adamski, Matthias H. Tschöp, Kristin Eckel-Mahan, Paolo Sassone-Corsi***

Previous studies revealed that circadian clocks are integral part of the regulation of biological processes in all tissues of organisms. Advanced knowledge of the molecular and cellular underpinnings of circadian biology indicated that circadian disruption can play a role in a wide range of pathologies. Metabolic diseases are often characterized by circadian misalignment in different tissues in organisms. However, how altered coordination and communication among tissue clocks relate to specific pathogenic mechanisms remains largely unknown. In this study we present a temporal and spatial atlas of circadian metabolism in the context of systemic energy balance and under chronic nutrient stress (high-fat diet [HFD]) by performing 24-hours metabolomics profiling of eight mouse tissues simultaneously. Suprachiasmatic nucleus, medial prefrontal cortex, gastrocnemius skeletal muscle, interscapular brown adipose tissue, epididymal white adipose tissue, liver, serum, and cauda epididymal sperm were collected every 4 hours across the light/ dark cycle from a single cohort of C57BL/6J mice after 10 weeks of ad-libitum access to standard chow or HFD. The metabolites in each sample were monitored and relatively quantified with UHPLC-MS/MS and GC-MS/MS. Comparative analysis reveals how the tissue metabolic pathways are linked and gated to specific temporal and spatial windows and how tissue-specific and inter-organ dynamic clocks emerge by nutrient challenge. Overall, we show in this study how dynamic metabolic relationships among tissues can be reconstructed across time and space and how integration of circadian metabolomics data from multiple tissues can improve our understanding of health and disease.

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BIOMEDICAL

P-174 Analytical pipeline to determine the healthy fecal metabolome in cryogenically collected samples

PRESENTING AUTHOR: ***Kajetan Trost, Steno Diabetes Center Copenhagen, Denmark***

CO-AUTHORS: ***Linda Ahonen, Tommi Suvitaival, Nina Christiansen, Trine Nielsen, Maja Thiele, Suganya Jacobsen, Aleksander Krag, Peter Rossing, Torben Hansen, Lars Ove Dragsted, Cristina Legido-Quigley***

Introduction: Fecal metabolomics is a valuable tool to discover links between fecal microbiota and disease and it is especially pertinent for research in gastrointestinal disorders, clinical nutrition and metabolic syndromes. Large interindividual differences exist in the fecal composition, however little is known about the actual within-sample heterogeneity in healthy sample donors. Methods: We collected and immediately froze one stool sample from 10 healthy volunteers and cryogenically drilled every sample in four areas along the specimen. We detected relatively polar metabolites by derivatization followed by two-dimensional gas chromatography and time of flight mass spectrometry, while we analyzed lipids using ultra high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry. Metabolite features with analytical variation above 30% were removed, the ratio of between subjects to within subject variation was used to assess sample homogeneity. Results: After exclusion of 2326 features that could not be annotated, we detected a total of 298 molecular features, of which 182 showed analytical variation x< 30%. Metabolites included amino acids, fatty acid derivatives, carboxylic acids and phenolic compounds. Lipids predominantly belonged to the groups of diacylglycerols, triacylglycerols and ceramides. As many as 79% (144 of 182) molecules showed less variability within the same individual than the variability observed between subjects. Conclusions: Feces show extensive biological variation, both within and between healthy individuals. Initial results show that molecules in abundance are those synthesized by the microbiome, ceramides, triacylglycerols and diacylglycerols.

P-175 Bariatric surgery impacts metabolism of branched chain amino acids and branched chain fatty acids

PRESENTING AUTHOR: ***Alicja Pakiet, University of Gdansk, Poland***

CO-AUTHORS: ***Maciej Wilczyński, Olga Rostowska, Justyna Korczyńska, Patrycja Jabłońska, Łukasz Kaska, Monika Proczko-Stepaniak, Piotr Stepnowski, Tomasz Śledziński, Adriana Mika***

Bariatric surgery is the most effective form of treatment of morbid obesity leading to substantial weight loss and remission of type 2 diabetes. In the targeted metabolomic analysis of serum amino acids by liquid chromatography–mass spectrometry (LC-MS) we investigated the effect of omega-loop gastric bypass (OLGB) in obese patients (n=50) at the time of the surgery and at 6-9 months after the surgery, and in lean controls (n=30). Obese subjects were characterized by elevated levels of branched chain amino acids (BCAA) – leucine and isoleucine, while post-OLGB patients exhibited reduced valine, leucine and isoleucine content. BCAA concentrations were positively correlated with marker HOMA-IR (R=0.3, p=0.007), proving the impact of BCAA levels on insulin resistance. Furthermore, we used gas chromatography–mass spectrometry (GC-MS) lipidomic approach to profile fatty acids in serum of study subjects. Statistical analysis showed significant increase in levels of several iso- and anteiso- (BCFA) in post-OLGB patients, which was negatively correlated with HOMA-IR (R=0.28, p=0.012). BCFA are beneficial to health due to their anticancer, immunosuppressive and antibacterial properties. Since BCAA can act as precursors for synthesis of BCFA, levels of mRNA of enzymes associated with BCAA catabolism were investigated. We found that visceral adipose tissue expression of branched-chain keto acid dehydrogenase E1 subunit beta (BCKDHB) and branched chain amino acid transaminase (BCAT) 1 and 2 was severely reduced in obese individuals. It is therefore possible that the improvement in levels of beneficial BCFA following bariatric surgery is a result of normalization of BCAA metabolism in adipose tissue.

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***AWARD WINNERS**

BIOMEDICAL

P-176 **Diet or surgery - A multi-platform metabolomics approach aiming to unravel dysregulated metabolic pathways in type 2 diabetes**

PRESENTING AUTHOR: ***Katharina Herzog, Lund University, Sweden***

CO-AUTHORS: ***Mahmoud Al-Majdoub, Johan Berggren, Andreas Lindqvist, Jan Hedenbro, Leif Groop, Nils Wierup, Peter Spégel***

Type 2 diabetes (T2D) is a devastating, chronic metabolic disease that presently affects 8.5% of the world's population, resulting in an unmet need for improved treatments. Roux-and-Y gastric bypass surgery (RYGB) is an effective approach to induce weight loss and T2D remission in obese individuals. The mechanisms driving T2D remission are, however, not fully understood. In addition, it is debated whether a low-calorie diet (LCD) may induce the same effects observed after RYGB. In this study, we used blood plasma from morbidly obese individuals with (n=10) or without T2D (n=9) to investigate the alterations in metabolite levels in response to LCD and subsequent RYGB. We applied three complementary metabolomics platforms based on ultrahigh-performance liquid chromatography (UHPLC) coupled to quadrupole time-of-flight mass spectrometry (QTOF-MS). Together, these platforms cover >300 identified metabolites, including a variety of lipids, low molecular weight metabolites, i.e. amino acids, and metabolites with intermediate polarity, i.e. acylcarnitines. After data processing using commercial and open-source tools and in-house libraries, we applied multivariate data analyses techniques such as linear mixed-effect regression models and orthogonal projections to latent structures effects projection (OPLS-EP), an approach that allows multivariate modeling of dependent samples. Our data suggest that the effects of LCD, and the combination of LCD and RYGB, elicit different changes in the fasting metabolic state. These effects were driven by changes in acylcarnitines and various lipids. Furthermore, analyzing metabolic profiles in response to mixed-meal tests identified differences between individuals with and without T2D, which may aid unraveling the mechanisms driving T2D remission.

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*AWARD WINNERS

BIOMEDICAL

P-177* Profiling the effect of Adult Onset Hypothyroidism (AOH) on the mouse heart in both sexes integrating untargeted GC- and LC-MS metabolomics

PRESENTING AUTHOR: ***Maria-Konstantina Ioannidi, Metabolic Engineering & Systems Biology Lab, Institute of Chemical Engineering Sciences, FORTH, Greece***

CO-AUTHORS: ***Marigoula Margarity, Maria I. Klapa***

Background: The present study is part of a multi-tissue investigation (brain, heart, liver) of the effect of Adult-Onset-Hypothyroidism (AOH) on the metabolic physiology of a chemical induction mouse model, intending to comparatively reconstruct the metabolic fingerprint of this pathophysiology at the organism level in both sexes. Here, we present the heart metabolic profiling, integrating gas and liquid chromatography-mass spectrometry (GC-/LC-MS)-based metabolomics with systems and network biology approaches. Methods: Tissue collection of 24 mice (male/female in sets of six euthyroid/hypothyroid) was performed on the 124th postnatal day, after applying an optimized tissue perfusion protocol with saline solution. An adapted to mouse heart metabolite extraction protocol was performed. Standardized data deposition and analysis was conducted using our software suite, M-IOLITE, and multivariate statistics tools. The differential metabolites were visualized within the mouse heart primary metabolism network that we have reconstructed from literature and our data. Results and Discussion: Indeed, the integration of GC-MS with LC-MS metabolomic analyses provided a broader overview of the mouse heart central carbon metabolism under AOH. Our results validated a lipid-centric metabolism for the heart, which becomes de-regulated under AOH characterized by decreased energy metabolism and TCA cycle activity. No extended inter-sex differences were observed, with the female exhibiting greater metabolic AOH insulation through an increased lipid oxidation activity. These results will be presented in comparison with the already acquired brain region metabolic profiles of the same animals towards a multi-tissue AOH metabolic physiology model. Acknowledgments: The work was supported by BITAD_MIS_5002469, ELIXIR-GR_MIS 5002780, EATRIS-GR_MIS 5028091, INSPIRED_5002550.

P-178 High-performance metabolomics diagnostic model for Non-alcoholic fatty liver disease (NAFLD)

PRESENTING AUTHOR: ***Takeshi Kimura, St. Luke's International University, Japan***

CO-AUTHORS: ***Masahiro Nojima, Yutaka Aoki, Kuniyoshi Hayashi, Hirotaka Fujimoto, Yuji Heike, Mariko Asami, Kazuhiko Suzuki, Kevin Urayama, Masaaki Matsuura, Takaaki Sato, Katsunori Masuda***

Non-alcoholic fatty liver disease (NAFLD) is an important disease due to its association with a broad range of disorders. We aimed to identify metabolome biomarkers for NAFLD using gas chromatography/mass spectrometry (GC/MS) and develop a diagnostic model for disease prediction. Methods: We enrolled 3,733 visitors of St. Luke International Hospital, Center for Preventive Medicine (Tokyo, Japan) between October 2015 and September 2016 who applied exclusion criteria for NAFLD diagnosis, e.g. daily alcohol intake (Men≥30g, Women≥20g), concurrent liver disease or malignancy, medication for metabolic diseases. Fatty liver was diagnosed by ultrasonography. Metabolome analysis was performed on blood serum samples using the GCMS-TQ8040 (Shimadzu, Japan). Results: The mean age of the participants was 51.8 years, and 826 (22.1%) were diagnosed with NAFLD. Compared to the non-NAFLD group, 57.0% of the metabolites (65/114 investigated metabolites) were significantly increased in the NAFLD population. The metabolite that demonstrated the strongest association was glutamic acid (P = 6.4 x 10-89 and AUC = 0.759), followed by 2-oxoglutaric acid and valine. These metabolites were significantly associated with NAFLD even after adjustment for sex, age, and BMI using logistic regression. Finally, using LASSO (least absolute shrinkage and selection operator), we constructed a diagnostic model including multiple metabolites (n=70), which achieved an AUC of 0.870. Discussion and Conclusions: We identified metabolites associated with NAFLD which were observed to be independent of obesity. A high-performance diagnostic model for NAFLD through integration of metabolomics analysis and machine learning was developed which may have potential for clinical application in the future.

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*AWARD WINNERS

BIOMEDICAL

P-179 Curcumin alleviates palmitate-induced inflammation and glycerolipid accumulation in C2C12 skeletal muscle cells

PRESENTING AUTHOR: ***Dean Ashley, University of Cambridge, United Kingdom***

CO-AUTHORS: ***McNally BD, Murfitt S, Middleton A, Sassano G, Russell P, Aleksic M, Griffin J***

Increased plasma concentrations of palmitate in obese individuals is suggested to enhance inflammation, lipotoxicity and insulin resistance in peripheral tissues, including skeletal muscle. Upon treatment with palmitate expression of the inflammatory cytokines Il6 and Tnfα were increased as measured by rtPCR, while co-treatment with curcumin ameliorated these increases. Published studies have shown that curcumin can increase AMPK activity in skeletal muscle of mice fed a high-fat diet, ameliorating inflammation, mitochondrial dysfunction and insulin resistance. However, the mechanism underpinning these changes has not been fully elucidated. As AMPK is an important regulator of lipid metabolism, we applied mass spectrometry to assess curcumin-induced alterations in the lipid profile of C2C12 myotubes exposed to 750 µM palmitate. Lipids were extracted using a modified Bligh and Dyer method, and lipid changes were assessed using open profiling C18-liquid chromatography-mass spectrometry. Treatment with palmitate raised concentrations of triacylglycerides (TAGs), diacylglycerides (DAGs), ceramides and phosphatidylcholine species. The elevated levels of TAGs and DAGs were reduced with curcumin co-treatment, while concentrations of other lipid species were unaffected. These results suggest the reduced inflammation and insulin resistance exerted by curcumin is driven by increased catabolism of DAGs and TAGs by alleviating the palmitate induced DAG activation of protein kinase C, JNK and IKK pathways.

P-180 Metabolomics as a diagnostic tool in inborn errors of metabolism

PRESENTING AUTHOR: ***Michel van Weeghel, Amsterdam UMC, Netherlands***

CO-AUTHORS: ***Mia L. Pras-Raves, Martin A. T. Vervaart, Angela C. M. Luyf, Lindsey Welling, Mendy Karssies, Sacha Ferdinandusse, Antoine H. C. van Kampen, Riekelt H. Houtkooper, Ronald J. A. Wanders, Annet M. Bosch, Hans R. Waterham, Frédéric M. Vaz***

Metabolomics involves the (semi-)quantitative and qualitative measurement of metabolites in biological matrices and is used as an analytical tool for biomarker discovery in research but have not frequently been applied to diagnostics. Inborn errors of metabolism (IEM) are routinely identified by targeted analysis of intermediary metabolites. Recent developments in high-resolution mass spectrometry, tracer-based metabolomics and ion mobility techniques, opens new avenues to perform (untargeted) metabolomics for the identification of new biomarkers in IEM. In our study, we investigated the metabolome profiles from a set of 120 plasma samples from patients with 10 known inborn errors of peroxisomal metabolism. We used reversed-phase, normal-phase, and HILIC chromatography followed by full-scan orbitrap-MS on the Q Exactive plus in positive and negative ionization mode and annotated over 200 polar metabolites and over a 1200 lipid species. We identified specific biomarkers for the different inborn errors of peroxisomal metabolism and discovered new potential biomarkers. Because this is a semi-targeted approach, identities of some of the newly found biomarkers remain to be characterized. Furthermore, we performed stable isotope tracer experiments on three classical and three variant (no illness at diagnosis by newborn screening, erythrocyte galactose-1-phosphate uridyltransferase activity 4-9%) galactosemia patients and were able to distinguish mild from severe patient (the galactosemia index), which ultimately could lead to personalized treatment of these patients. The ultimate goal is to use this type of metabolomics analysis for first line screening of IEMs.

P-181 Metabolic Signature Associated with Insulin Sensitivity Improvement Following a Weight Loss Intervention

PRESENTING AUTHOR: ***Jarlei Fiamoncini, University of São Paulo - School of Pharmaceutical Sciences, Brazil***

CO-AUTHORS: ***Milena Rundle, E. Louise Thomas, Barbara Gelhaus, Ronny Scheundel, Alexander Haag, Christian Hoffmann, Guus Roeselers, Denise Sonntag, Jimmy D Bell, Gary Frost, Hannelore Daniel***

Metagenomics and metabolomics analysis are allowing the exploration of the connection between intestinal microbiota and host metabolism. Within the NutriTech project, 72 healthy subjects (male and female, ± 60 y.o.) were enrolled in a weight loss intervention (20% dietary energy restriction for 12 weeks), losing in average 6.4% of their body weight. In some volunteers, weight loss was associated with an improvement in insulin sensitivity, while in others only minor changes in insulin sensitivity were observed, despite weight loss. Looking for metabolic signatures that could help understand these outcomes, two PLS-DA models were built: one using the results of body composition and metabolite profiling of plasma samples collected during an OGTT and a mixed meal test; the second using data from intestinal microbiota composition. Marker metabolites of both groups were identified: in particular urea, specific bile acids, amino acids and acylcarnitines, as well as several discriminant bacterial species. Individuals without improvement in insulin sensitivity following the weight loss, displayed higher plasma concentrations of tauroursodeoxycholic acid (a taurine-conjugated bile acid), had higher proportions of Bilophila and other urease-positive bacteria within their microbiome, but lower concentrations of urea and deoxycholic acid. The differences in microbiota composition are in line with the differences in plasma urea and bile acids concentrations. This analysis brings new phenotypic information associated with differential weight loss and allows the generation of hypothesis. Further studies are warranted and validation of the findings in larger cohorts is of course necessary.

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BIOMEDICAL

P-182 Batch independent screening of Inborn Errors of Metabolism with untargeted metabolomics

PRESENTING AUTHOR: ***Michiel Bongaerts, Erasmus Medicale Centre, Netherlands***

CO-AUTHORS: ***Ramon Bonte, Serwet Demirdas, Henk Blom, George Ruijter***

It has been demonstrated that untargeted metabolomics can be used for the screening of Inborn Errors of Metabolism (IEM). However, most studies have used control samples from the same experimental run for determining Z-scores of metabolites. This limits both the amount and matching (sex, age) of the controls with the patient of interest. We explored the use of controls originating from different experimental runs / batches. In this way a better reference population can be defined for a given patient. 6 batches were independently processed and subsequently merged. In total 195 controls and 33 patients were measured including 20 different IEM's. We explored multiple normalization methods to correct for signal drift and batch effects. Internal standard based normalization methods performed better than statistical based methods. We developed a normalization method, Metchalizer, which uses a mixed effect model and internal standards for normalization. This method showed the best performance on our data compared with other methods. After normalization with Metchalizer 56 out of 69 biomarkers for patients with known IEM's were detected by using a reference population from other batches. This study identified multiple age/ sex related metabolites by using a regression model. The variance in control samples is determined by both biological and technical variance. The biological variance for a given metabolite might strongly depend on age and/or sex and therefore matching patient and reference is important when determining Z-scores. We show that using controls from other batches can solve this issue as long as batch effects can be corrected.

P-183 Metabolomic alterations in hypocholesterolemic drug users

PRESENTING AUTHOR: ***Minako Matsumoto, Keio University, Japan***

CO-AUTHORS: ***Sei Harada, Miho Iida, Suzuka Kato, Ayako Kurihara, Ayano Takeuchi, Kazuyo Kuwabara, Daisuke Sugiyama, Tomonori Okamura, Toru Takebayashi***

BACKGROUND: Although hypocholesterolemic drugs are the first-line therapy for cardiovascular disease prevention, the most highly used stains are known for their various adverse effects such as muscle inflammation, liver damage, and hyperglycemia. This study sought to determine variations of plasma metabolite concentrations related to hypocholesterolemic drugs intake using capillary electrophoresis-mass spectrometry. METHODS: All data and samples were collected from participants of the Tsuruoka Metabolomics Cohort Study which was conducted in Japan. Information on medical history, lifestyle, and medications was collected through a standardized, self-administered questionnaire. After excluding those with a history of cancer, stroke, and myocardial infarction, we selected participants on hypocholesterolemic drugs alone as drug users (n=385) and those with LDL-cholesterol 140 mg/dl or above but without any drug as controls (n=1143). Associations between the drug intake and plasma metabolite concentrations were examined by linear regression. Then, analysis of co-variance was conducted to examine the association between levels of fasting plasma glucose (FPG) and hypocholesterolemic drug-related metabolites. RESULTS: Out of 115 polar metabolites analyzed, five were identified as hypocholesterolemic drug-related metabolites. These associations were independent of sex, age, systolic blood pressure, FPG, smoking, alcohol intake, physical activity, dietary energy, and liver function. Three out of five metabolites (betaine, threonine, and cystine) were included in the trans-sulfuration pathway. Cystine was also found to be associated with elevated levels of FPG, which may reflect the effect of statin intake on hyperglycemia. CONCLUSION: Alterations of plasma metabolite levels in transsulfuration pathway were observed with hypocholesterolemic drug intake.

P-184 Metabolomics study on the effect of combination treatment between pioglitazone and piceatannol on Non-Alcoholic Fatty Liver Disease in Rats

PRESENTING AUTHOR: ***Shun Wan Chan, Technological and Higher Education Institute of Hong Kong, Hong Kong***

CO-AUTHORS: ***Shun-Wan Chan, Chi-On Chan, Tung-Ting Sham, Huan Zhang, Yam-Fung Ng, Daniel Kam-Wah Mok***

Non-alcoholic fatty liver disease (NAFLD) is one of the most common liver disorders worldwide. NAFLD prevalence increases recently and becomes an important public health concern. Given the importance of insulin resistance in the pathogenesis of NAFLD, insulin sensitizers were broadly investigated in treating NAFLD. However, the long-term usage related side effects of insulin sensitizers have limited its utility. Combination drug therapy utilizes more than one medication but each agent is given at a dose much lower than the normal therapeutic dose (i.e., minimal side effects are anticipated) resulting in synergistic therapeutic outcomes. In this study, we used metabolomics approaches to investigate a novel drug therapy using pioglitazone (a commonly used insulin sensitizer) in combination with piceatannol, a natural analog of resveratrol, to treat NAFLD in rat fed with high fat diet (HFD). Our in vivo study clearly demonstrated that Sprague-Dawley rats fed with HFD for 4 weeks could establish NAFLD. Treatment with pioglitazone in combination with piceatannol would could significantly reduce the adverse effects induced by HFD. It could lower the liver's lipid content and the total cholesterol level in serum. It was found that the metabolomics profile of combination drug therapy group was significantly different from that of the HFD group but it was getting closer to that of the control group. Additionally, more than 20 metabolites were found as potential biomarkers indicating that this combination drug therapy could effectively modulate the lipid metabolism and result in improvements in NAFLD.

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BIOMEDICAL

P-185 In vivo metabolic profiling in brown and white adipose tissue using open flow microperfusion (OFM)

PRESENTING AUTHOR: ***Elmar Zügner, Joanneum Research Forschungsgesellschaft mbH, Austria***

CO-AUTHORS: ***Simon Schwingenschuh, Thomas Altendorfer-Kroath, Sonja Kainz, Yasemin Erdem, Günther Rauter, Petra Kotzbeck, Joanna Hummer, Thomas Birngruber, Christoph Magnes***

One of the major challenges for the healthcare system is the increasing prevalence of obesity. Several therapeutic approaches to overcome obesity modify adipose tissue metabolism to induce sustained weight loss. However, how treatment effects are mediated on multiple metabolic pathways remain elusive. We present a novel method to investigate tissue-specific metabolism in brown and white adipose tissue (BAT, WAT) in rats. We performed open flow microperfusion (OFM) allowing collection of interstitial fluid of BAT and WAT, and subsequent metabolic profiling of the collected samples. Metabolic profiling was performed in OFM samples with volumes as small as 15 µl each. Analyzation was done using HILIC-HRMS (Vanquish UHPLC, QExactive mass spectrometer Thermo Fischer Scientific) in positive and negative electro spray ionisation mode with a gradient elution and a run time of 37 minutes. Metabolic profiling identified 141 metabolites including lipids, fatty acids or amino acids that were classified into metabolites usable for multivariate (MVA) or for univariate (UVA) data analysis. 94 MVA metabolites showed a median standard deviation of peak intensity in pooled quality control samples of 7.1%, low ppm and no retention-time deviation, making them usable for principal component analysis (PCA) and analysis of variance (ANOVA). UVA metabolites were only usable for ANOVA. PCA showed a distinct signature for 2 metabolite classes in WAT and BAT samples. MVA and some UVA metabolites from these classes also showed statistically significant differences in the ANOVA. These results serve as a proof-of-concept to directly investigate adipose tissue metabolism in-vivo.

P-186 Liquid chromatography-mass spectrometry-based approach to evaluate lipidomic changes of plasma in Yijin-Tang-treated obese mice

PRESENTING AUTHOR: ***Jueun Lee, Korea Basic Science Institute, South Korea***

CO-AUTHORS: ***So Min Lee, Eunjung Kang, Hye-Lin Kim, Jeeyoun Jung, Geum-Sook Hwang***

Obesity is one of the most widespread health problems and is characterized by an aberrant lipid metabolism. The herbal remedy Yijin (Erchen)-tang (YJT) is widely used to treat obesity-related disorders. However, the change of lipid metabolism by YJT still remains unknown. In this study, liquid chromatography (LC)-mass spectrometry (MS)-based approach was applied to investigate specific changes in lipid metabolism associated with the therapeutic effects of YJT-treated obese mice. C57BL/6N mice were fed a high-fat and high-cholesterol (HFHC, 40% fat and 1% cholesterol) diet for 8 weeks and treated them with YJT for an additional 6 weeks. We then performed untargeted lipidomic analysis of plasma using UPLC-QTOF MS coupled with multivariate statistical analysis. Partial least squares-discriminant analysis score plots showed that YJT altered the lipid metabolic pattern of HFHC mice. In particular, ceramides and triglycerides with saturated fatty acids and monounsaturated fatty acids were significantly changed by YJT, which were significantly associated with insulin resistance, the AGE-RAGE signaling pathway in diabetic complications and adipocytokine signaling pathway in pathway enrichment analysis. In addition, the changes of total WBC, CRP and blood glucose, and correlation analysis between lipids and those clinical markers showed the effect of YJT treatment on ameliorating inflammation and improving insulin resistance. These data suggest that YJT ameliorates obesity-induced systemic inflammation and insulin resistance by regulating lipid metabolism, and demonstrated that lipidomic profiling is a useful method to investigate the therapeutic effects of herbal decoctions in traditional Korean and Chinese medicine.

P-187 Anabolic androgenic steroids induce weight loss in male Wistar rats in relation to free fatty acids in plasma

PRESENTING AUTHOR: ***David Balgoma, Uppsala University, Sweden***

CO-AUTHORS: ***Sofia Zelleroth, Alfhild Grönbladh, Mathias Hallberg, Curt Pettersson, Mikael Hedeland***

The abuse of anabolic androgenic steroids (AASs) in supratherapeutic doses is a source of public concern. Among other effects, AASs affect lipogenesis and lipolysis in different tissues. Lipidomics has the potential of unraveling the mechanism of the effects of AASs in health and disease. Consequently, we treated Wistar rats every third day with vehicle, nandrolone decanoate, and testosterone undecanoate (supratherapeutic doses). The weight of the animals was measured every third day and, on day 18, the animals were euthanized, blood was collected, and plasma isolated. After extraction, the plasma lipidome was analyzed on an Acquity UPLC-Synapt G2S Q-ToF equipped with a BEH C18 column. Free fatty acids showed a specific regulation in relation to the treatment with AASs. They increased in the treatment with nandrolone decanoate, but they did not change with testosterone undecanoate. Weight decreased with the treatment with nandrolone decanoate but weight did not change with testosterone undecanoate. As free fatty acids in plasma are known to reflect the levels of triacylglyceride lipolysis in the adipose tissue, weight regulation is associated with the levels of free fatty acids in plasma. This effect might play a role in the effect of androgens in health and disease.

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BIOMEDICAL

P-188 Development of a New Diagnostic Tool for the Early Detection of Type 2 Diabetes (DeTecT2D)

PRESENTING AUTHOR: ***Rui Wang-Sattler, Helmholtz Zentrum München, German Research Center for Environmental Health, Germany***

CO-AUTHORS: ***Stefan Brandmaier, Isobel D. Stewart, Andreas Lechner, Norbert Stefan, John Chambers, Peter Bergsten, Therese Koal, Christian Gieger, Jerzy Adamski, Annette Peters***

It is alarming that 1 in 2 people (212 million) with diabetes were undiagnosed according to the International Diabetes Federation. One reason for this problem is that the only sensitive assay for the early detection of Type 2 Diabetes (T2D) is an oral glucose tolerance test (OGTT), which is time consuming and costly. The goal of the H2020 EIT Health funded DeTecT2D consortium therefore is to develop a new metabolomics-based assay for the sensitive detection of pre-diabetes and T2D from a single fasting blood sample. A set of 3 metabolite biomarkers for pre-diabetes and T2D was previously identified by members of the DeTecT2D consortium. Based on clinical studies, the consortium extended this set to 6 metabolites, which were further evaluated in two population-based human cohorts (n=9870). In KORA and Fenland studies, the set of biomarkers showed comparable AUC values (83% and 94%) to detect individuals with pre-diabetes and newly diagnosed T2D, respectively. We further evaluated whether the set of 6 metabolites was capable of replacing the standard OGTT. Based on individuals with normal fasting glucose, we obtained AUC values of 74% and 77% for pre-diabetic and diabetic individuals in the KORA S4 study, respectively. Although our extended set of biomarkers does not outperform the OGTT, it is highly predictive for newly diagnosed T2D. Thus, our set of biomarkers holds great promise for use as a routine screening tool for individuals with pre-diabetes and undiagnosed T2D.

P-189 Mimicking protein restriction by inhibiting SLC6A19 (B0AT1) —a potential target to treat metabolic disorders

PRESENTING AUTHOR: ***Kiran Javed, Australian National University, Australia***

CO-AUTHORS: ***Stefan Broer***

Recent studies have established that dietary protein restriction has beneficial impacts on the metabolic health and it promotes improved glucose homeostasis by increasing the induction of FGF21 from liver. B0AT1 (SLC6A19) is the major neutral amino acid transporter in the lumen of the intestine that carries out the bulk of amino acid absorption from the diet. It also reabsorbs neutral amino acids in proximal tubule of the kidney. Mice lacking B0AT1 show signs of protein restriction such as elevated levels of FGF21 and reduced mTORC1 activity. Moreover they have improved glucose homeostasis and are protected from diet induced obesity, making it a potential target to treat metabolic diseases such as phenylketonuria and type 2 diabetes. We determined the postprandial levels of amino acids and other metabolites under low, standard and high protein diet of SLC6A19ko, wt and hz mice. All essential amino acids showed a significant positive correlation with the protein content of the diet in WT mice. The neutral AAs that requires SLC6A19 for its absorption were reduced in the blood of SLC6A19ko mice whereas other amino acids showed no significant difference as compared to WT mice. The reduced levels of neutral AA were more prominent under conditions of high protein diet. We also found a few bacterial metabolites of AA fermentation that were correlating with the protein content of the diet in the blood of SLC6A19ko mice. This study highlights the potential of this transporter as a target to induce protein restriction for the treatment of metabolic disorders.

P-190 A Reliable Mass Spectrometry Methodology for Podocyturia Evaluation in Genetic and Non-Genetic Kidney Diseases

PRESENTING AUTHOR: ***Christiane Auray-Blais, Université de Sherbrooke, Canada***

CO-AUTHORS: ***Tristan Martineau, Michel Boutin, Anne-Marie-Côté, Bruno Maranda, Daniel Bichet***

Increased podocyte levels in urine (podocyturia) is an early sign of kidney abnormalities in patients. Currently, kidney damages are evaluated by using proteinuria measurements and the estimated glomerular filtration rate (eGFR) which, in some cases, might not always be efficient for early detection of patients. Moreover, most analytical techniques for the evaluation of podocyturia are tedious, time-consuming, and may lead to marked results variability. The primary objective of this research project was to develop and validate a multiplex, quantitative ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) approach to evaluate two proteins related to podocyturia, podocalyxin and podocin in urine samples. The secondary aim targeted the analysis of these biomarkers in samples from women with preeclampsia, Fabry disease patients, and related gender-matched controls. One mL of a random urine sample was centrifuged, followed by the addition of cleavable peptide standards to the supernatant. Trypsin digestion was performed (2h at 37oC) and the peptides were purified by solid-phase extraction and evaporated. A 10-min UPLC-MS/MS was developed and validated. Normal values were established. Our results show that women with preeclampsia had abnormal urine levels of both proteins with a higher sensitivity for podocalyxin. Fabry male patients presented a slight elevation of podocin whereas untreated Fabry females had increased concentrations of both podocalyxin and podocin. Correlations were established with these biomarkers and some clinical parameters. This reliable methodology might be an efficient tool for early diagnosis of women with preeclampsia and Fabry disease patients. It is also applicable to other genetic and non-genetic kidney diseases.

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BIOMEDICAL

P-191 Fecal metabolome and microbiota composition in obese Göttingen minipigs as influenced by source of carbohydrates

PRESENTING AUTHOR: ***Mihai Victor Curtasu, Aarhus University, Denmark***

CO-AUTHORS: ***Valeria Tafintseva, Zachary Bendiks, Maria L. Marco, Achim Kohler, Yetong Xu, Helle Nygaard Lærke, Knud Erik Bach Knudsen, Mette Skou Hedemann.***

The objective of this study was to explore the effects of ad libitum feeding of two high-fat diets supplemented with either high amylose maize starch (HIMA) or fructose on the fecal metabolome and microbiota composition. These effects were examined in obese Göttingen Minipigs as a model for human metabolic disorders. Thirty Göttingen Minipigs were fed with two experimental diets for five months, and fecal material was sampled three times during the dietary intervention. Non-targeted liquid chromatography-mass spectrometry was used to explore metabolite differences, short-chain fatty acids (SCFAs) were quantified by gas chromatography, and bacterial diversity was assessed by 16S rRNA gene sequencing. Sequencing data were analyzed using QIIME 2, and together with metabolomics and SCFAs data a sparse multi-block partial least squares regression model was calculated. Higher proportions of Bacteroidetes were found in the fecal contents of animals fed HIMA compared to that fed fructose together with higher quantities of total organic acids and total SCFAs. Furthermore, concentrations of acetate and acetate+propionate+butyrate were positively associated with the Ruminococcus genus and increased levels of several dicarboxylic acids (2,4-dimethyladipic acid, 3,3-methylglutaric acid) and pantothenic acid. Minipigs fed fructose contained higher butyrate levels, which were closely associated with enrichments in Roseburia, Clostridiaceae, Turicibacter, and Ruminococcus (Lachnospiraceae family). Bacteria from the Coprococcus, Blautia or Coprococcus genera were associated with a higher intensity of enterolactone (m/z 297.1135; [M+Cl] m/z 333.0902). Overall, we observed that different carbohydrate sources induced a contrasting stimulation of the microbial taxa which further reflected in a divergent fecal metabolome profile.

P-192 Analysis of bile acid profiles in patients with nonalcoholic fatty liver disease (NAFLD) depending on NAFLD severity

PRESENTING AUTHOR: ***Youngae Jung, KBSI, South Korea***

CO-AUTHORS: ***Won Kim, Geum-Sook Hwang***

Nonalcoholic fatty liver disease (NAFLD) is one of common causes of chronic liver disease that involve hepatic accumulation of triglycerides. NAFLD is progressed from a nonalcoholic fatty liver (NAFL) to nonalcoholic steatohepatitis (NASH). Bile acids has been considered to be related with pathogenesis of NAFLD. In this study, we analyzed the profile of bile acids in NAFLD patients using LC-MS system. Sera acquired from 234 Asian subjects with biopsy-proven NAFLD and metabolically healthy controls were used for analysis of bile acids. Bile acid profiles included total 13 bile acids such as cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), hyodeoxycholic acid (HDCA), ursodeoxycholic acid (UDCA), glycocholic acid (GCA), glycochenodeoxycholic acid (GCDCA), glycodeoxycholic acid (GDCA), taurodeoxycholic acid (TCA), taurochenodeoxycholic aicd (TCDCA), taurodeoxycholic acid (TDCA), taurohyodeoxycholic acid (THDCA), tauroursodeoxycholic acid (TUDCA). Most of bile acids showed the significant increases in NASH compared to NAFL, whiles didn't show the significant changes in NAFL compared to No-NAFLD. And primary bile acids were mainly increased in NASH. Our results showed that the bile acid profiles in NAFLD patients were changed depending on NAFLD severity and also presented to differently be changed according to types of bile acids.

P-193* Plasma metabolomics identifies potentials biomarkers in different stages of Non-alcoholic fatty liver disease

PRESENTING AUTHOR: ***XIANGPING LIN, Laboratoire CSPBAT - Paris 13 University - UFR SMBH, France***

CO-AUTHORS: ***Xinyu LIU, Mohamed TRIBA, Zhicheng LIU, Laurence LE MOYEC, Marianne ZIOL, Nada HELMY, Corinne VONS, Nadia BOUCHEMAL, Guowang XU, Carina Prip-buus, Philippe SAVARIN***

Non-alcoholic fatty liver disease (NAFLD), characterized by abnormal accumulation of triglycerides (TGs) in the liver, which is becoming the most common chronic liver diseases in the global adult population. A previous study found that plasma metabolome was a better predictor for steatosis (80%) than noninvasive basal clinical data (58%). In this study, we investigate whether High-resolution mass spectrometry-based Plasma metabolomics could identify potentials biomarkers in different stages of the disease, and explore the further molecular mechanism involved in the progression of NAFLD. Plasma were collected from obese subjects with Normal liver (n=19), Steatosis (n=39) and NASH (n=24). To cover polar and non-polar metabolites, samples analysis were performed using two separated ultra-performance liquid chromatography High-resolution mass spectrometry-based Metabolomic and lipidomic platform, respectively. Compared with Normal liver obese, Steatosis or NASH subjects had abnormal liver function, high plasma TGs concentrations and were insulino-resistance. Binary Logistic Regression analysis found each a panel of potentials biomarkers for Steatosis, NASH and Steatosis from NASH, the regression models were evaluated by internal validation with Area under the ROC Curve around 0.7 (mean for validation). The small sample size may be a limit of this study, still, our results suggested that High-resolution mass spectrometry-based Plasma metabolomics may be a promising way to identify plasma biomarkers of NAFLD, the external validation is in progress, after validated in other independent studies, these biomarkers might help to diagnosis Steatosis from NASH, monitor development of NAFLD and shed light further on molecular mechanism involved in disease progression.

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BIOMEDICAL

P-194 Metabolomics approaches to metabolic changes after the intermittent fasting diet

PRESENTING AUTHOR: ***Jeongae Lee, Reaserch Scientist, South Korea***

CO-AUTHORS: ***Yoon Hwan Kim, Kyoung Heon Kim, Bong Chul Chung, Young-Ran Yoon, Ji-Won Lee***

Human serum with intermittent fasting was analyzed by metabolomics and multivariate analysis, and increased of 2-hydroxyphenylacetate and decreased of phenylpyruvate were observed in phenylalanine metabolism.

P-195 The impact of ulcers on the metabolomic profile of serum of diabetic patients

PRESENTING AUTHOR: ***Arsenty Melnikov, International Tomography Center SB RAS / Novosibirsk State University, Russian Federation***

CO-AUTHORS: ***Yuriy Tsentalovich, Vadim Yanshole***

It is believed that the alternations in lipid and protein catabolism in patients with diabetes mellitus (DM) leads to various complications, such as diabetic ulcer. Despite the fact that diabetic ulcer is one of the most common complication of DM, it is still poorly studied. Current study is devoted to the comparative metabolomic analysis of serum obtained from patients with type 2 DM with and without ulcers. To obtain the serum metabolomic profiles, two high-performance liquid chromatography methods (HILIC and RPLC) in conjunction with high-resolution ESI-q-TOF mass-spectrometric detection were used. This approach made possible to register more than 1700 features (in both HILIC and RPLC data), yielding more than 250 metabolites and lipids. For the majority of metabolites, their levels in the serum of diabetic patients with and without ulcers are similar, with few exceptions including 1,5–anhydroglucitol (fold change = 3.2, AUC = 0.96 ± 0.02), bilirubin (fold change = -3.1, AUC = 0.89 ± 0.03) and a group of phospholipids with the mean fold change equals to -1.48 ± 0.02. For all listed differences the p-value is lower than 0.05. This study shows that the development of diabetic ulcer is accompanied by the decrease in the concentrations of phospholipids and some lipid-related metabolites. Acknowledgements: Supported by the RFBR (18-33-20097) and by RFBR and NSO (18-415-543006).

P-196 Fast detection of drugs and metabolites in urine by Flow Injection Analysis coupled to Magnetic Resonance Mass Spectrometry

PRESENTING AUTHOR: ***Christopher Thompson, Bruker Daltonics Inc., United States***

CO-AUTHORS: ***Matthias Witt, Aiko Barsch, Markus Godejohann***

A fast method for detection of drugs and their metabolites in urine using flow injection analysis (FIA) and magnetic resonance mass spectrometry (MRMS) is presented. Roughly 250 samples can be measured in 24h using this technique. Pooled urine samples were purified by SPE using Merck LiChrolutEN SPE cartridges. Samples were extracted with methanol from SPE cartridges and diluted in Methanol for FIA. Each sample was analyzed in 5 minutes by FIA-MRMS in ESI in positive and negative ion mode. Spectra were acquired in a mass range m/z 107 – 3000 in quadrupolar detection mode with a resolving power of 1,350,000 at m/z 200. For the final mass spectrum 28 single scans were averaged. Analysis of data was performed with MetaboScape 4.0. The data of the ESI(+) and ESI(-) were combined for feature analysis. More than 2100 features were found for the pooled urine samples. More than 90% of the detected features could be assigned with a molecular formula. 300 drug candidates were annotated in the urine samples by matching a target list of expected compounds derived from HMDB (http://www.hmdb.ca/) with a mass error tolerance of only 0.5 ppm. The detected drugs were compared with the medication of the patients. Several drugs were found only in specific pooled urine samples. By comparing the relative abundances of features of all samples, possible metabolites of drugs could be identified.

P-197 Improved identification of human CSF metabolites using workflow-based approaches allowing integration of multiple search engines

PRESENTING AUTHOR: ***Kim Kultima, Uppsala University, Sweden***

CO-AUTHORS: ***Payam Emami Khoonsari, Christoph Ruttkies, Stephanie Herman, Kristian Peters, Ralf Weber, Thomas Lawson, Joachim Burman, Ola Spjuth, Steffen Neumann and Kim Kultima and members of the ELIXIR Implementation Study on Metabolite Identification***

Untargeted metabolomics as a high-throughput molecular phenotyping technique is growing across all domains in the life sciences. The data processing and analysis is often performed using different tools with little standardization and automation for interoperable and reproducible research. We have previously developed a comprehensive and interoperable workflow that integrates all necessary components for doing mass spectrometry-based identification and quantification of metabolites as well as the downstream analysis1–3. To improve metabolite identification we have now developed a workflow approach that allows integration of MS1 and MS2 information, resulting in improvement in statistical scoring of the identified compounds as well as a substantial decrease in computation time. The workflow includes multiple search engines such as CSI:FingerID and MetFrag. The workflow presented can directly connect to the MetaboLights data repository, and performs identification as well as quantification, alignment, and matching using a large collection of available tools. Together, we achieved a complete integration of several major metabolomics software suites resulting in a turn-key workflow for mass spectrometry-based metabolomics. This approach vastly improved identification of metabolites expressed in human cerebrospinal fluid (CSF). The workflow is available on Galaxy, Nextflow and Pachyderm on PhenoMeNal. This study is part of the European collaborative ELIXIR Implementation Study on Metabolite Identification.

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BIOMEDICAL

P-198 DecoMetDIA: Deconvolution of Multiplexed MS/MS Spectra for Metabolite Identification in SWATH-MS based Untargeted Metabolomics

PRESENTING AUTHOR: ***Yandong Yin, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, China***

CO-AUTHORS: ***Zheng-jiang Zhu***

With recent advances in mass spectrometry, there is an increased interest in data-independent acquisition (DIA) techniques for metabolomics. With DIA technique, all metabolite ions are sequentially selected and isolated using a wide window to generate multiplexed MS/MS spectra. DIA strategy enables a continuous and unbiased acquisition of all metabolites and increases the data dimensionality, but presents a challenge to data analysis due to the loss of the direct link between precursor ion and fragment ions. Currently, only few DIA data processing methods are developed for metabolomics application, including MS-DIAL and MetDIA. Nevertheless, there is still a lack of software tools to support the SWATH-MS based untargeted metabolomics. Here we presented a new software tool, namely, DecoMetDIA, to support metabolite identification in SWATH-MS based untargeted metabolomics with spectra deconvolution with extracted MS2 EICs to satisfy the need of data processing tools for SWATH-MS technique in metabolomics. With the large-scale metabolite annotation of the MS2 spectra deconvoluted by DecoMetDIA using MetDNA and Sirius/CSI:FingerID, we found that about 70~80% features were annotated, which proved that the MS2 spectra deconvoluted by DecoMetDIA were of high accuracy and ready to be used for large scale metabolite identification.

P-199 Automated microfluidic cell culture of stem cell derived dopaminergic neurons

PRESENTING AUTHOR: ***Edinson Lucumi Moreno, Leiden University, Netherlands***

CO-AUTHORS: ***Khalid I. W. Kane, Siham Hachi, Moriz Walter, Javier Jarazo, Miguel A. P. Oliveira, Thomas Hankemeier, Paul Vulto, Jens C. Schwamborn, Martin Thoma, Ronan M. T. Fleming***

Parkinson's disease is a slowly progressive neurodegenerative disease characterised by dysfunction and death of selectively vulnerable midbrain dopaminergic neurons and the development of human in vitro cellular models of the disease is a major challenge in Parkinson's disease research. We constructed an automated cell culture platform optimised for long-term maintenance and monitoring of different cells in three dimensional microfluidic cell culture devices. The system can be flexibly adapted to various experimental protocols and features time-lapse imaging microscopy for quality control and electrophysiology monitoring to assess cellular activity. Using this system, we continuously monitored the differentiation of Parkinson's disease patient derived human neuroepithelial stem cells into midbrain specific dopaminergic neurons. Calcium imaging confirmed the electrophysiological activity of differentiated neurons and immunostaining confirmed the efficiency of the differentiation protocol. This system is the first example of an automated Organ-on-a-Chip culture and has the potential to enable a versatile array of in vitro experiments for patient-specific disease modelling.

P-200 Comprehensive Analysis of Metabolites Using GC-MS/MS and LC-MS/MS -An Application to the Research of the Intestinal Environment

PRESENTING AUTHOR: ***Akihiro Kunisawa, Shimadzu Corporation, Japan***

CO-AUTHORS: ***Akihiro Kunisawa, Takanari Hattori, Shuichi Kawana, Shin-ichi Kawano, Yoshihiro Hayakawa, Junko Iida, Eiichiro Fukusaki, Mitsuharu Matsumoto.***

A part of the metabolites produced by intestinal microbiota are absorbed constantly from the intestinal lumen and carried to systemic circulation; and play a direct role in health and disease. Recently, increasing attention has been devoted to the relationship among intestinal microbiota, the metabolites, and health and disease. There are limited reports concerning the function of metabolites produced by intestinal microbiome. Furthermore, these studies are targeting specific metabolites such as short-chain fatty acids but not global metabolites (metabolome). In this study, we analyzed metabolites in mice feces using GC-MS/MS and LC-MS/MS for comprehensive analysis. As results of GC-MS/MS analysis for the extracts of the mice feces, 100 metabolites were detected. Main compounds of 100 metabolites were short-chain fatty acids, organic acids and sugars. Seventeen sugars that are difficult to analyze using LC-MS/MS were detected. As results of ion-pairing LC-MS/MS analysis, 17 metabolites which were mainly amino acids were detected. As results of non- ion-pairing LC-MS/MS analysis, 75 metabolites were detected. Main compounds of 75 metabolites were amino acids, nucleotides, nucleosides and organic acids. These results indicated that comprehensive analysis of metabolites using GC-MS/MS and LC-MS/MS is effective for the research of the intestinal environment. In addition, we also report on the interpretation of the metabolome data using the Shimadzu Multi-omics Data Analysis Pack, which will facilitate the examination of the results.

POSTER SESSIONS 1 AND 2 – Monday and Tuesday – all odd number posters will be on display.
POSTER SESSIONS 3 AND 4 – Wednesday and Thursday – all even number posters will be on display.

*AWARD WINNERS

BIOMEDICAL

P-201 Metabolomic characteristics of the facial skin in young and aged cohorts from a Caucasian female population

PRESENTING AUTHOR: ***Philipp Ternes, BASF Metabolome Solutions GmbH, Germany***

CO-AUTHORS: ***Allison Guinta, Nicolas Del Bene, Neethu Abraham, Lauren Junker, Wendy Chan, Hunter Cameron, Anita Samuga, Nengyi Zhang, George Kritikos, Vimal Rawat, Girish Srinivas, Sabrina Letoy-Okombi, Valérie André***

An in-depth understanding of the changes which are occurring on multiple levels in the human skin during aging is of major interest for the development of novel cosmetic ingredients. Aging of the facial skin is known to be accompanied by profound changes in the skin microbiota and in biophysical skin quality parameters (e.g. Jugé et al. 2018, J. Appl. Microbiol. 125, 907; Zouboulis et al. 2018, Mech. Ageing Dev. 170, 98). To gain a better understanding of the interactions between the skin and its microbiota, we have conducted a simultaneous analysis of the skin microbiome and metabolome in the crow's feet/undereye area in cohorts of ≈ 50 young (age 18–35 years, wrinkle grade 0–1) and ≈ 50 aged (age > 55 years, wrinkle grade 5–6) healthy Caucasian women. This poster presentation will focus of the metabolomic characteristics of these two cohorts.

P-202 Simultaneous quantification of tryptophan pathway metabolites in serum, plasma and faeces using UHPLC-MS/MS method

PRESENTING AUTHOR: ***Dai Long Vu, Max Delbruck Center for Molecular Medicines, Germany***

CO-AUTHORS: ***Andras Balogh, Hendrik Bartolomaeus, Jennifer Kirwan***

Tryptophan and its metabolites play key roles in heart and brain health. The eventual fate of tryptophan involves an interplay between host and gut microbiome. Quantification of the tryptophan pathway metabolites provides important information about the eventual fate of tryptophan in the body which may be important for the study of certain diseases. Although current methods exist for the quantification of a subset of these metabolites, methods for simultaneous quantification of most of these compounds are still missing. We demonstrate a robust, accurate, reproducible method for the quantification of 30 compounds related to the tryptophan pathway using an ultrahigh performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) technique. The analytical method was fully validated for serum, plasma and faeces, following European Medicines Agency guidelines. We report on the analytical linearity, limit of detection, limit of quantification, matrix effect, recovery, accuracy and precision of the method. In addition, stability of standards and samples were also investigated. Except for tryptophan which was degraded after 24 hours at 4 oC, all other tested compounds were stable up to 48 hours, exceeding the run length of a typical batch. Our study also offers a high throughput analytical method as the separation in a reversed phase system can separate the analytes in a 10 minute gradient, fostering the analysis of batches with high number of samples.

P-203 Effect of nutrition on host-gut microbiota metabolic interactions of preterm infants fed with own mother´s milk and donated mother milk

PRESENTING AUTHOR: ***José david Piñeiro, Neonatal Research Group, Spain***

CO-AUTHORS: ***Anna Parra-Llorca, María Gormaz, María Cernada, Guillermo Quintás, María Carmen Collado, Máximo Vento, Julia Kuligowski***

Breastfeeding is the gold standard for nutrition of the term and preterm infant. For preterm infants whose mothers are unable to provide of own mother's milk (OMM), pasteurized donor human milk (DHM) is the preferred alternative. Here, the metabolic signature of preterm infants has been studied to unscramble the effect of nutrition on their metabolism. Urine samples of 40 preterm infants fed with OMM (N=20) and DHM (N=20) aged between 2 and 4 weeks of life were collected and analysed employing Liquid Chromatography-High Resolution Mass Spectrometry. A peak table was extracted with XCMS and a total of 2241 m/z features were retained after data pre-processing and filtering. Pathway analysis (MetaboAnalyst) revealed a significant alteration of three pathways related to the biosynthesis of hormones. Significant differences were found between urinary fingerprints of preterm infants receiving OMM and DHM (Partial Least Squares Discriminant Analysis, p-value=0.009). 73 features were annotated corresponding to a total of 25 metabolites. Seven putatively annotated metabolites were related to the activity of the gut microbiome. This is the first study of the urinary metabolome of preterm infants fed with DHM vs. OMM. Observed changes could be potentially attributed to i) diet-dependent changes in the colonization pattern of preterm infants that might affect hormone production, ii) changes in the hormone levels in human milk and/or iii) the effect of pasteurization on DHM hormone levels. Based on the results, future studies for assessing the impact of maternal nutrition as well as treatment and storage on human milk composition are encouraged.

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*AWARD WINNERS

BIOMEDICAL

P-204 Metabolomic evaluation of probiotic beneficial effect on non-alcoholic fatty liver diseases in mouse model using LC-Orbitrap MS and GC-TOF MS

PRESENTING AUTHOR: ***Jeong Seok Yu, Kookmin University, South Korea***

CO-AUTHORS: ***Dae Hee Han, Ki-Tae Suk and Do Yup Lee***

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver diseases caused by numerous factors such as genetics, dysbiosis, and diet. NAFLD is defined as from mild liver steatosis to non-alcoholic steatohepatitis (NASH). NASH may lead to liver cirrhosis and hepatocellular carcinoma. Recent studies have proposed dysbiosis as the critical factor for NAFLD. Dysbiosis causes endothelial barrier dysfunction and leads to leaky gut. Compromised gut barrier allows the translocation of bacteria and bacteria-derived molecules to liver through portal vein. Colonic homeostasis is closely related with intestinal short chain fatty acids (SCFAs) which are the fermentation end-products by gut microbiota. Especially, butyrate is the most important energy source for enterocytes and contributes to colonic homeostasis. In current study, we applied four different combination of mass-spectrometric platforms to comprehensively measure a range of metabolic features and some key known factors. We first evaluated pharmabiotic effect on the mice fed with western-diet by oral administration of 6 probiotics. Indeed, we observed the coordinated changes in SCFAs levels from cecal samples. Based on multivariate statistical model, we putatively identified metabolites indicating strong positive correlation with the SCFA alteration. Finally, we monitored the modulation of primary and secondary bile acids that may provide detailed mechanistic in molecular dynamics between gut and liver.

P-205 Paroxetine treatment in mice increases fecal bile acid levels that correlate with behavior

PRESENTING AUTHOR: ***Frederik Dethloff, MPI of Psychiatry, Germany***

CO-AUTHORS: ***Elijah Emmanuel, Vargas Fernando, Quinn Robert, Herzog David, Mueller Marianne, Dorrestein Pieter, Turck Christoph***

Selective Serotonin Reuptake Inhibitors (SSRIs) are commonly used drugs to treat major depressive disorder. Since SSRIs are drugs that are taken orally it is conceivable that they can affect gut microbiota and their functions and thereby impact other body organs. In this regard it has now become increasingly clear that microbiota communicate with the central nervous system through the gut-brain-axis. Several reports have shown that a distorted gut microbiota composition that is mediated by its secreted molecular constituents can impact the central nervous system and contribute to the development of psychiatric disorders. We treated DBA/2J mice for 2 weeks with the SSRI paroxetine and assessed their behavioral response with the forced swim test (FST) and the light-dark box test (LDB). In order to investigate paroxetine's effects on the gut microbiota we analyzed fecal pellets as a proxy. In addition to 16S rRNA we interrogated by tandem mass spectrometry the metabolite profiles of fecal pellet extracts. Untargeted metabolite profiling of drug- and vehicle-treated mice revealed altered levels of features that belong to the bile acid class and have a strong correlation with behavioral measures. No significant alterations of the taxa occured in response to the two week paroxetine treatment. Microbiota-derived metabolites may constitute biomarkers able to predict paroxetine treatment response.

P-206 'Functional Microbiomics' – Assessing Nutrition-Microbiome-Host Interaction in Blood and Feces

PRESENTING AUTHOR: ***Barbara Wolf, BIOCRATES Life Sciences AG, Austria***

CO-AUTHORS: ***Hai Pham Tuan, Ulf Sommer, Svenja Heischmann, Doreen Kirchberg, Xenia Iwanowa, Radu Talmazan, Martin Buratti, Therese Koal***

In recent years, microbiome research has dramatically reshaped our understanding of how microbes impact on a multitude of (patho-)physiological processes in the host. However, causal links are still lacking to a large extent. Metabolomics allows the investigation of microbial metabolic activities, and is thus the ideal technology to assess functional nutrition-microbiota-host crosstalk. Here, we discuss the application of a newly developed targeted assay for the quantification of endogenous and microbiota-derived metabolites. 10 µl human plasma and fecal samples were analyzed by a standardized, quantitative assay in kit format allowing for the analysis of 630 metabolites. 106 small molecules from 13 compound classes are analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS), hexoses and 523 lipids from 12 lipid classes by flow-injection analysis-tandem mass spectrometry (FIA-MS/MS) on an Agilent 1290 Infinity UHPLC – SCIEX QTRAP® 5500. MetIDQ™ software was used for the entire automated workflow. In plasma, more than 455 metabolites were quantified above LOD with high, and more than 120 metabolites in fecal samples. To a large extent, the small molecules and lipids quantified in feces overlap with those in plasma. A higher number of lipids, especially phosphatidylcholines and triglycerides, were quantified in plasma compared to fecal samples. In addition to endogenous metabolites, a multitude of microbiota-derived metabolites were quantified. The capability to quantify microbiota-derived metabolites in blood and fecal samples allows for correlation studies also with data from other omics technologies to investigate functional nutrition-microbiome-host interplay for uncovering causal links to pathophysiological processes, disease development, and response to drug treatment.

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*AWARD WINNERS

BIOMEDICAL

P-207 Developing an optimal fecal extraction protocol to maximize metabolite coverage for metabolomics studies

PRESENTING AUTHOR: *Mary Boyce, Edith Cowan University, Australia*

CO-AUTHORS: *Jessica Pandohee, Claus T Christopherson, David I Broadhurst*

Background: Metabolomics is the analysis and unbiased relative quantification of all metabolites in a biological sample. In order to study the entire metabolome of a biological system, it is essential that most, if not all, of the metabolites present in the system in question are extracted. Human stool is a complex solid sample making reproducible extraction of metabolites challenging. The aim of this work was to develop extraction methods that maximize the metabolite coverage for untargeted and semi-targeted metabolomics studies. Methods: To investigate the effect of solvent polarity on the metabolite extraction coverage, homogenised fecal samples (100 mg) were individually extracted with 200 L solvent. The supernatant was dried and the residue derivatised using methoxyamine in pyridine followed by MSTFA. The samples were analysed on a ThermoFisher Q-Exactive GC-MS. The metabolites were identified using both our in-house library of 400 metabolites and NIST. Results: Of the 16 solvents tested, water (pH 2), methanol and acetone extracted 91, 102 and 91 respectively of the 400 analytes in our in-house library with RSD <30%. The solvents MTBE and chloroform extracted 28 and 30 analytes, respectively. When performing a biphasic extraction using water (pH 2):methanol:acetone (40:30:30) as aqueous layer and MTBE as non-aqueous layer, 99 and 40 metabolites with an RSD < 30% are identified respectively. On the other hand, 54 (non-aqueous) and 130 (aqueous) metabolites are identified in a sequential extraction. Conclusions: A sequential extraction involving MTBE followed by a methanol/acetone/water phase provides optimal coverage of feces for untargeted or semi-targeted studies.

P-208 Can probiotics reduce urogenital malodour in women? – A targeted metabolomics approach

PRESENTING AUTHOR: *Scarlett Puebla-Barragan, The University of Western Ontario, Canada*

CO-AUTHORS: *Justin Renaud, Mark Sumarah, Gregor Reid*

Millions of women around the world seek medical attention due to urogenital malodour, which negatively impacts their quality of life. The main causes are urinary tract infections (UTIs) and bacterial vaginosis (BV), which can occur as a result of vaginal dysbiosis, defined as a disruption of the vaginal microbiota. Unfortunately, the standard treatment for these conditions is a course of antibiotics, which is not always effective and takes several days to improve the symptoms; therefore, a more efficient way to reduce malodour is required. Previously, it was reported that the biogenic amines: putrescine, cadaverine, trimethylamine, and tyramine, are responsible of malodour in BV. However, it is not clear if these are also present in higher concentrations during a UTI. Using HILIC-MS analysis and a targeted metabolomics approach, we identified that uropathogenic strains of E. coli (which cause around 80% of UTIs) can significantly produce putrescine, cadaverine, and trimethylamine when using female urine as substrate in vitro. These results were confirmed analyzing 30 clinical samples. This demonstrates that there is an overlap in the causes of malodour during a UTI and BV. With the same analytical approach, we demonstrated that certain lactobacilli strains can degrade these malodorous compounds in conditions that simulate the vaginal environment in vitro. Some lactobacilli probiotics are already known to help in restoring the vaginal microbiota. In addition to this benefit, our evidence suggests that some lactobacilli strains could also target urogenital malodour – making them a more efficient alternative to the current treatment.

P-210 Analytical methods to measure kinetics of fermentation of non-digestible carbohydrates inside the human gut using novel gastrointestinal sampling capsules

PRESENTING AUTHOR: *Melany Rios-Morales, University Medical Center Groningen, Netherlands*

CO-AUTHORS: *Mara van Trijp, Theo Boer, Rebecca Heiner-Fokkema, Albert Gerding, Fjodor Sluijs, Guido Hooiveld, Barbara Bakker, Dirk-Jan Reijngoud*

Consumption of non-digestible carbohydrates (NDC) has been linked to many health benefits. However, detailed knowledge of the exact fate and impact of NDC in the intestinal tract is lacking. Gut microbiota use NDC as substrates to produce short chain fatty acids (SCFAs) and other metabolites. In mice after feeding NDC, only the uptake flux of SCFAs from the intestine correlated with improvements of the metabolic syndrome. In humans, access to this inner world is now possible by an orally swallowable gastrointestinal capsule, that can deliver or sample at an exact location in the gastrointestinal tract. Specific challenges of using the gastrointestinal capsules are: 1) The small volumes of sample obtained. To overcome this, we optimized the analytical protocols to measure NDC breakdown, gut microbiota composition, and bacterial fermentation products (notably SCFAs) in the same small and complex samples as we expect to obtain from the capsule; and 2) the capsule with sample remains in the body until its excretion. Hence, we developed a stabilizing solution to be preloaded in the capsule that can efficiently stop fermentation for up to 2 days. This will allow us to collect a representative sample of the human colon. Furthermore, we verified that the quench solution was not a source of variation in the analytical analyses mentioned above. This analytical toolbox to use gastrointestinal sampling capsule systems will allow us to study fibre intake, SCFAs uptake fluxes and improvements of the metabolic syndrome markers in human subjects.

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*AWARD WINNERS

BIOMEDICAL

P-211 Metabolomics of Intestinal Bacteria for Novel Kidney Stone Therapeutics

PRESENTING AUTHOR: *Casey Chamberlain, University of Florida, United States*

CO-AUTHORS: *Marguerite Hatch, Timothy J. Garrett*

Kidney stone disease afflicts 1 in 11 people in the U.S. and burdens the economy with an annual treatment cost of over $10 billion. Nearly 80% of kidney stones are composed of calcium oxalate (CaOx) and form when humans are exposed to oxalate through the diet and endogenous glyoxylate metabolism in the liver. Recently, significant clinical interest has turned to the intestinal microbiome, specifically to a class of “oxalotrophic” bacteria which degrade dietary oxalate. These bacteria reduce the oxalate absorbed by the body, which directly influences CaOx stone formation risk. The commensal Oxalobacter formigenes (Oxf) utilizes oxalate as its sole energy source and has the exclusive ability among oxalotrophs to draw endogenous circulating oxalate from the bloodstream into the intestine to increase oxalate availability, allowing it to feed on both metabolic and dietary oxalate. Hence, its use as a probiotic therapy for kidney stone disease is of key interest. Compelling evidence demonstrates that Oxf produces an unidentified secreted bioactive compound to regulate oxalate transport proteins in the intestinal wall to bring oxalate across the epithelium. This compound, if identified, could potentially serve as a future therapy for kidney stones and other oxalate-derived conditions. This study seeks to identify this secreted factor and other oxalate disease-relevant metabolic products through a UHPLC-HRMS-based characterization and comparison of the metabolome and lipidome of several strains of Oxf. We also present a related analytical investigation of Lactobacillus acidophilus and Lactobacillus gasseri, two additional intestinal oxalotrophs that also show promise as future oxalate-degrading probiotic therapies.

P-212 Comparing metabolomic profiles of plasma, urine, feces and food in monkey study

PRESENTING AUTHOR: *Suzumi M Tokuoka, The University of Tokyo, Japan*

CO-AUTHORS: *Megumi Ishibashi, Fumie Hamano, Yoshihiro Kita, Yoshiya Oda*

Human metabolome profiles are affected by environment factors, especially lifestyle such as eating habits and exercise. We examined systematically whether metabolomic profiles would be similar in the model animals under the same environmental conditions even though genetic backgrounds are different. We chose monkey as a model animal that seems to be closer to humans than rodents. The mass spectrometric measurements of hydrophilic and hydrophobic metabolites were performed using both non-targeted and targeted approaches on 20 cynomolgus monkeys, which were composed of four groups in each five animals according to birthplace, age and health condition. The metabolomic profiles of serum, urine and feces were differed widely among individuals rather than the groups despite being kept for a long period under the same environment and food. No marked difference in profiles was found between chronically sick group (with diarrhea and vomiting) and others, but some metabolite levels may be changed in sick monkeys. In spite of the food does not contain arachidonic acid and contains very few docosahexaenoic acid, significant amount of them were detected in plasma. Those levels were different among monkeys and fatty acid profiles in plasma may vary by a country of origin. Phospholipid profile was characteristic among plasma, urine and feces. Plasma contains more phosphatidylcholine with unsaturated longer fatty chain, but urine contains shorter fatty chain and lysophospholipid rather than diacyl phospholipids. Feces have more sphingomyelin and phosphatidylethanolamine. We are now looking for relationships of metabolic profiles among plasma, urine, feces and food.

P-213 Immunomodulatory Function of Human Gut Microbiota during Early Life Development

PRESENTING AUTHOR: *Zdenek Spacil, Masaryk University, Czech Republic*

CO-AUTHORS: *Veronika Vidová, Eliška Stuchlíková, Anne-Christine Aust, Jana Klanová, Zdeněk Spáčil*

The exposome paradigm represents a complement to heritable factors in the understanding of health and disease. While the human gut microbiota is considered a pivotal external factor affecting the metabolism and overall health of the host through immunomodulation. The early life stimulation shaping the innate immune system may be the initial mechanism behind the development of some diseases. An appropriate multi-omics toolbox is required to explore immune-metabolic viewpoint of microbial immunomodulation and disease. We applied tandem mass spectrometry assays using a triple quadrupole mass analyzer (selected reaction monitoring – SRM) for metabolic profiling of specific catabolites of tryptophan and kynurenine pathways in biofluids and cell culture material. SRM-proteomics assays were used to determine circulating and local inflammatory markers. High resolution/accurate mass (HR/AM) exploratory mass spectrometry (Orbitrap Fusion, Thermo Scientific) was utilized for metabolic screening. Results demonstrated microbial metabolites clinically relevant markers of inflammation, dysbiosis or a disease condition, documenting a tight connection between microbial colonization and the host immune system. We have mapped the distribution of microbiota-associated metabolites within biofluids in adults, pregnant women, and neonates and linked them to levels of acute phase proteins. The study is pioneering the functional characterization of microbiota, mediated via stimulation of the immune system and essential to understanding its role in human health. This is consistent with age-dependent changes, underlying emerging concepts of exposome and inflammaging. Funding: GACR project (17-24592Y), GAMU project (MUNI/G/1131/2017), CETOCOEN PLUS (MEYS, CZ.02.1.01/0.0/0.0/15_003/ 0000469), the RECETOX research infrastructure (MEYS, LM2015051; CZ.02.1.01/0.0/0.0/16_013/0001761).

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*AWARD WINNERS

BIOMEDICAL

P-214 Surveying the spectrum of bacterial sialic acids using high resolution mass spectrometry

PRESENTING AUTHOR: ***Hugo Kleikamp, TU Delft, Applied Sciences, Biotechnology, Netherlands***

CO-AUTHORS: ***Yuemei Lin, Mark van Loosdrecht, Martin Pabst***

Over the past decades nonulosonic acids have been increasingly found in (pathogenic) prokaryotes, decorating the contact surface to their external environments. A recent phylogenetic large-scale study discovered a surprisingly wide distribution of an ancient core nonulosonic acid biosynthetic pathway. However, predicted ORFs often do not encode for functional entities, and most importantly, genomics alone does not reveal the important post synthesis processing events, which make nonulosonic acids so unique. There are many open questions surrounding the evolution and utilisation of nonulosonic acids in prokaryotes, as well as their role in diseases. Unfortunately, their analysis with conventional methods is a laborious process. Therefore, we developed a fast but highly specific assay for the screening of existing nonulosonic acids, and the discovery of novel nonulosonic acid variants, using high-resolution mass spectrometry, in combination with a specifically developed data processing tool. The high specificity of the assay allows for direct analysis from crude cell lysates, and analysis of low abundant species in complex communities. The application of high-resolution mass spectrometry also enables stable isotope incorporation experiments to follow novel biosynthetic routes in bacteria. Using this newly developed assay, we provide a comparative large-scale study on the diversity and distribution of different classes of nonulosonic acids across a variety of species, including purely environmental samples. The developed assay serves as a tool to explore complex samples and communities to study the broader ecological context of this prominent, but still poorly analysed class of sugars.

P-215 Metabolic insights from plasma samples of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) patients

PRESENTING AUTHOR: ***Arnaud Germain, Cornell University, United States***

CO-AUTHORS: ***Arnaud Germain, David Ruppert, Susan M. Levine, Maureen R. Hanson***

The well-documented constellation of debilitating symptoms endured by Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) patients implicates a severe disruption of homeostasis of metabolic networks. Here we reflect on the knowledge generated by the plasma datasets issued from our three cohorts totaling 113 distinct female subjects, 47 controls and 66 patients. The chemical fingerprint of our first cohort consisted of 361 metabolites, and conclusions from our quantitative analysis focus on disturbances in fatty acid and lipid metabolism. For our second cohort, the Metabolon® platform expanded our vision to 832 data points divided in eight super-pathways and imply a redox imbalance in disease symptomatology. Our latest dataset consists of 768 metabolic measurements from Metabolon®'s global metabolomics technology, while their complex lipid panel quantified 1,022 lipids. The concentrations of a few dipeptides were negatively affected in the patient cohort, indicating a potential disruption in protein catabolism, while differences in abundances of certain triacylglycerols (TAGs) were also detected. These molecules are fatty acids, which are required for various bodily functions, acting as the most important caloric source of energy homeostasis in mammals, serving as a flexible on-demand energy depot. TAGs are also an integral part of the bloodstream where they facilitate absorption of nutrient molecules such as fat-soluble vitamins. Abnormally high levels have, however, been linked to a wide range of cardiovascular problems. Our studies represent valuable steps in understanding the underlying problems that manifest in patients affected by this disease of unknown cause and poor prognosis.

P-216 Effects of dipeptidyl peptidase 4 inhibitor on the human urinary metabolomic profiling

PRESENTING AUTHOR: ***Jihyun Kang, Seoul National University, South Korea***

CO-AUTHORS: ***Bora Kim, Yun Kim, SeungHwan Lee, Kyung-Sang Yu, In-Jin Jang, Joo-Youn Cho***

Dipeptidyl-peptidase IV inhibitor (DPP-4) is oral medication for type 2 diabetes mellitus that stimulates insulin secretion by inhibiting degradation of the incretins, glucagon-like peptide-1 and glucose-dependent insulinotropic peptide. Therefore, DPP-4 inhibitors maintain blood glucose homeostasis by suppressing glucagon secretion and slowing gastric emptying. In this study, we aimed to discover sets of biomarkers for effects of DPP-4 inhibitors in urine samples from healthy volunteers and explore its underlying mechanism of action using metabolomics approach. Urine samples from 34 healthy subjects treated with DPP-4 inhibitor were collected at baseline and after 7 days treatment period and analyzed using Ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS) metabolomics platform. A total of 4775 metabolic features were detected through UPLC-QTOF-MS and putative markers were identified (FDR adjusted $P<0.0005$, power >0.9995). After treatment of DDP-4 inhibitor, healthy subjects showed relative intensity increase in 9 endogenous metabolites, dihydroxyfumaric acid, tyrosylthreonine, glutamine, leucylproline, ethylglycine, threonic acid, n-methyl-l-glutamic acid, methionylalanine, and kynurenic acid. 5 endogenous metabolites, including histidinal, arabinonic acid, phenylalanylalanine, alanylhydroxyproline, and capryloylglycine were significantly down regulated after treatment. Among the putative markers, 5 dipeptides showed differences after treatment. DPP-4 preferentially cleaves Xaa-Pro or Xaa-Ala dipeptides from the N-terminus of polypeptides and preventing the degradation of incretin hormones, by DPP-4 inhibition, speculated relative intensity of dipeptides change in postdose. In this study, pharmacometabolomics provides powerful tools for identifying effects of global metabolomics profiling by DPP-4 inhibitor treatment.

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*AWARD WINNERS

BIOMEDICAL

P-217 Determination of metabolic profiles of serum samples from women with PCOS based on LC-MS/GC-MS techniques

PRESENTING AUTHOR: ***Anna Stefaniak, Medical University of Gdańsk, Department of Biopharmacy and Pharmacodynamics, Poland***

CO-AUTHORS: ***Buszewska-Forajta Magdalena, Szybiak Aleksandra, Kowalewska Agnieszka, Rachoń Dominik, Markuszewski Michał Jan***

Despite such a high incidence, the pathogenesis of polycystic ovary syndrome still remains unexplained. PCOS is multifactorial disorder, which is diagnosed in about 10% of women of reproductive age. It is connected with ovulatory dysfunction and polycystic ovaries appearance and also with higher level of androgens concentration. Therefore, PCOS is one of the main reasons of female infertility. Besides to endocrine dysfunction, it may cause the development of different metabolic disorders. The use of the metabolomic approach in a field of PCOS aims to identify specific metabolic pathways potentially involved in the pathophysiology of this frequent disorder. Determination and comparison of the metabolic profiles of serum samples obtained from women with PCOS and healthy controls was the main aim of this study. Untargeted metabolomic analysis was conducted with the use two complementary analytical techniques: liquid chromatography and gas chromatography coupled with mass spectrometry. Next step was identification metabolites specific for biochemical pathways disturbed in PCOS. Univariate and multivariate statistical analysis showed the difference of metabolic profiles in studied groups of women. During the untargeted metabolomic analysis of serum samples, a few metabolites connected with metabolic disorders of amino acids, carbohydrates, steroid hormones, lipids, purines and citric acid cycle were identified. Understanding the pathomechanism of this syndrome and the identification of new potential biomarkers of PCOS will contribute to its early recognition and effective treatment.

P-218 Metabolic characterization and predictive model construction for partial hepatectomy mortality in porcine model

PRESENTING AUTHOR: ***Hyung Do Kwon, Kookmin University, South Korea***

CO-AUTHORS: ***Do Yup Lee***

The molecular and cellular mechanism in liver regeneration process following partial hepatectomy has been well known; however, hepatectomy mortality still remains significant problem in clinics. Therefore, we explored metabolic features reflected in serum metabolite after partial hepatectomy in porcine model. Pig shares the similarity in anatomy and physiology with human so it has been regarded as a proper model for liver study. In this study, based on gas chromatography time-of-flight mass spectrometry (GC-TOF-MS), primary metabolite profile was acquired from three groups, sham, 70%, and 90% liver resection groups. Un-supervised multivariate statistics revealed distinctive profiles of the three groups. And subsequent modeling based on orthogonal projection in latent structure-discriminant analysis (OPLS-DA) proposed key molecular features that discriminated hepatectomy mortality. The linear combination with malic acid and methionine showed the best predictive power in which the area under the curve (AUC) ranged from 0.939 to 0.989. The current study provides the understanding of liver regeneration and metabolic capacity impaired by hepatectomy.

P-219 Metabolomic profiling of orbital lymphoproliferative diseases

PRESENTING AUTHOR: ***Eri yamaguchi, Tokyo Medical University, Japan***

CO-AUTHORS: ***Eri Yamaguhci, Hiroyuki Shimizu, Yasuko Aita, Atsumi Tomita, Yoshihiko Usui, Hiroshi Goto, Masahiro Sugimoto.***

Orbital lymphoproliferative diseases, particularly mucosa-associated lymphoid tissue (MALT) lymphoma and IgG4-related ocular disease (IgG4-ROD), have similar clinical and also histopathological features, and are therefore often difficult to differentiate. In this study, we conducted metabolomic profiling of IgG4-ROD and MALT lymphoma tissue samples. Six samples of orbital MALT lymphoma (mean age 62.3 years; 4 males and 2 females) and 11 samples of IgG 4-ROD (mean age 64.8 years, 4 males and 7 females) and their matched controls samples were included. Liquid chromatography with time-of-flight mass spectrometry (LC-TOF-MS) was used for profiling lipid soluble metabolites. Principal component analysis clearly showed four groups: adipose tissue, tumor tissue, IgG4-ROD, and MALT lymphoma. Comparison between orbital adipose tissues and matched samples showed significant differences in 174 and 132 metabolites in IgG4-ROD and MALT lymphoma, respectively. Comparison between IgG4-ROD and MALT lymphoma showed significant differences in 12 metabolites. These tissue-specific difference would help understanding the pathogenesis of these diseases.

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*AWARD WINNERS

BIOMEDICAL

P-220 Metabolomics in Nonhuman Primate Models for Radiation Biodosimetry in Emergency Preparedness

PRESENTING AUTHOR: ***Albert Fornace, Jr., Georgetown University, United States***

CO-AUTHORS: ***Evan L. Pannkuk, Evagelia C. Laiakis, Kirandeep Gill, Shreyans K. Jain, Khyati Y. Mehta, Denise Nishita, Kim Bujold, James Bakke, Janet Gahagen, Simon Authier, Polly Chang, Albert J. Fornace Jr.***

Rapid assessment of radiation signatures in noninvasive biofluids may aid in assigning proper medical treatments for acute radiation syndrome (ARS) and delegating limited resources after a nuclear disaster. Metabolomic platforms allow for rapid screening of biofluid signatures and show promise in differentiating radiation dose and time post-exposure. Here, we use global metabolomics to differentiate temporal effects found in nonhuman primate (NHP) urine and serum small molecule signatures after a 4 Gy total body gamma irradiation. Random Forests analysis show days (1 – 60) after a 4 Gy exposure can be readily separated and provide critical information for ARS treatment. Eight compounds involved in protein metabolism, fatty acid β-oxidation, DNA base deamination, and general energy metabolism were identified in each biofluid. The greatest perturbations were seen at 1 d in urine highlighted by increases in carnitine, acylcarnitines, xanthine, creatine, 7-methylguanine, and dimethylarginine. Changes in serum metabolite concentrations were more dynamic (1 – 21 d) including carnitine, acylcarnitines, guanine, oleamide, and hypoxanthine. We also developed a targeted multiplex assay validating a six compound panel (hypoxanthine, carnitine, acetylcarnitine, proline, taurine, and citrulline) previously identified in a training cohort at 7 d after a 4 Gy exposure. Receiver operating characteristic (ROC) curve analysis indicated the highest sensitivity and specificity for carnitine and acetylcarnitine (urine, area under the curve [AUC] = 0.99) and taurine, carnitine, and hypoxanthine (serum, AUC = 0.95). These results highlight the utility of mass spectrometry (MS) platforms to differentiate time post-exposure and acquire reliable quantitative biomarker panels for classifying exposed individuals.

P-221 Effect of repeated freeze-thaw cycles on NMR measured lipoproteins and metabolites in biofluids

PRESENTING AUTHOR: ***Guro Giskeødegård, NTNU, Norway***

CO-AUTHORS: ***Feng Wang, Julia Debik, Trygve Andreassen, Leslie R. Euceda, Tonje H. Haukaas, Hartmut Schäfer, Tone F. Bathen***

Metabolic profiling of biofluids by Nuclear Magnetic Resonance (NMR) spectroscopy serves as an important tool in disease characterization, and its accuracy largely depends on the quality of the samples. We aimed to explore possible effects of repeated freeze and thaw cycles (FTCs) on concentrations of lipoprotein parameters in serum and metabolite concentrations in serum and urine samples. Serum and urine samples were collected from two sets of 20 healthy donors, and aliquots were subjected to 1–5 FTCs. Samples were subsequently analyzed by NMR spectroscopy, and lipoprotein parameters and metabolites were quantified using a commercial analytical platform. In total, 112 lipoprotein parameters, and 20 serum and 35 urine metabolites were quantified. Principal component analysis showed no systematic changes related to FTCs. Samples from the same donor were closely clustered, showing a higher between-subject variation than within-subject variation. The coefficients of variation were small (<2.1%, <1.4% and <2.2% for lipoprotein parameters, and serum and urine metabolites, respectively), showing a low variation in samples from the same donor. Systematic effects of FTCs on the concentrations were tested by Wilcoxon tests between consecutive FTCs. Only five lipoprotein parameters (VLCH, VLFC, V1CH, V4CH, and V3FC) differed significantly between FTC 3 and 4 (p-values<0.05), while other lipoprotein and metabolite concentrations showed no significant change. In conclusion, five FTCs did not significantly alter the concentrations of serum and urine metabolites, and introduced only minimal changes to serum lipoprotein parameters evaluated by the NMR- based platform.

P-222 Lipidomic analysis of acellular cod skin xenograft versus traditional dermal substitutes

PRESENTING AUTHOR: ***Aristotelis Kotronoulas, Center for Systems Biology, University of Iceland, Iceland***

CO-AUTHORS: ***H. S. Jónasdóttir, R. S. Sigurðardóttir S. Halldórsson, G. G. Haraldsson, Óttar Rolfsson***

Acellular fish skin (ACS) has emerged as a dermal substitute used to promote wound healing. The effects of ACS have been associated to the poly-unsaturated fatty acids (PUFA) although their presence in processed ACS has not yet been determined. Here, we compared the total free fatty acid (FFA) and lipid profiles of ACS to that of human cadaver skin (HCS) and two grafts of bovine origin. FFA quantification was performed by GC-FID and the lipid profile analysis by untargeted UPLC-MS/MS. Our results showed that ACS contained significantly higher omega3 FAs and PUFA than all other tested grafts, with percentages that exceeded 20% and 30% of the total FAs content respectively. The majority of the PUFAs found in HCS were omega-6 while the two grafts of bovine origin presented insignificant PUFA amounts. Regarding the grafts´ lipidomic profile, both unsupervised PCA and supervised PLS analysis demonstrated that all analyzed materials had very different lipid composition. Based on the heatmap and the hierarchical clustering, approximately 50 lipids from positive and negative ESI mode were identified as the most distinctive between the four materials and their possible structure was annotated. Further analysis of the lipid profiles demonstrated that phosphatidylcholine containing 20:5 and 22:6 omega3 PUFAs were characteristic for the ACS grafts. Our study showed that the lipid and fatty acid content of the ACS is different to other wound grafting materials. The high omega3 PUFA found may be associated with a range of positive wound healing mechanisms including anti-inflammatory activity, cell migration and stimulation.

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BIOMEDICAL

P-223 Improving the Diagnosis, Treatment, and Prevention of Chronic Kidney Disease by Establishing a Mass Spectrometric Reference Measurement Procedure for Parathyroid Hormone

PRESENTING AUTHOR: ***Candice Ulmer, Centers for Disease Control and Prevention, United States***

CO-AUTHORS: ***Hubert Vesper***

Parathyroid hormone (PTH) is a key biomarker for chronic kidney diseases, one of the leading health conditions in the USA. In addition, the measurement of parathyroid hormone (PTH) along with the respective fragmented versions of this hormone in serum and/or plasma is necessary for the detection, diagnosis, and prevention of calcium, phosphate, and vitamin D disorders. Current quantitation approaches suffer from sensitivity, PTH instability, and method measurement variability. CDC's PTH standardization program is improving the accuracy and reliability of measurements performed in patient care. A reference measurement procedure was developed for the quantitation of intact PTH (1-84) and select PTH fragments in serum. Stable isotope labeled internal standards were spiked prior to sample preparation. PTH isolation was optimized using immunocapture methodologies with monoclonal antibodies. The UHPLC-HRMS-based reference procedure allows for the highly specific detection and quantitation of intact PTH (z=6) without isotopic interference from the labeled internal standard. Preliminary results demonstrate the ability to quantify intact PTH and PTH fragments within a 5-ppm mass accuracy window without enzymatic digestion. Calibration curves using stable isotope labeled internal standards were generated for iPTH, N-terminal PTH fragments, C-terminal PTH fragments, and mid-region PTH fragments. Initial assessments demonstrate excellent linearity (R2 = 0.991-0.997), reproducibility, and sufficient sensitivity of the UHPLC-HRMS system with a linear range of 15-3000 pg/mL PTH. This method was applied to samples from healthy patients and patients with different CKD stages. PTH and its fragments were detectable in these samples.

P-224 Metabolic profiling of renal cystic fluids in autosomal dominant polycystic kidney disease patients

PRESENTING AUTHOR: ***Woori Chae, Seoul National University, South Korea***

CO-AUTHORS: ***Hyunjin Ryu, Seung Seok Han, Joo-Youn Cho***

Autosomal dominant polycystic kidney disease (ADPKD) is a genetic disorder resulted from mutations in PKD1 and PKD2 genes. It occurs in about 1 in 400 to 1000 and is the fourth leading cause of end-stage renal disease. Tolvaptan, a vasopressin receptor antagonist, is the first line treatment for ADPKD but there are various known side effects. Currently, no drug has been developed to inhibit the formation and development of polycysts. In this study, we compared the metabolic profiles of renal cyst fluids obtained from patients with ADPKD and those with simple cysts as a control to find a new therapeutic target. Differences in metabolite profiles according to various clinical parameters were also compared in ADPKD patients. Fifteen cyst fluids from ADPKD patients and twenty-seven cyst fluid from patients with simple cysts were collected and analyzed with both reverse phase and hydrophilic interaction liquid chromatography column using ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry. Though we did not find any significant differences between ADPKD and simple cyst groups, several statistically significant metabolites were found in ADPKD patients according to the incidence of advanced chronic kidney disease. It was the first report of comparative human metabolome profile of ADPKD and simple cyst fluids, which may contribute to understanding the pathogenesis of ADPKD.

P-225 Analysis of low-molecular-weight fraction of honeybee venom using mass spectrometry-based methodologies

PRESENTING AUTHOR: ***Agnieszka Klupczynska, Department of Inorganic and Analytical Chemistry, Poznan University of Medical Sciences, Poland***

CO-AUTHORS: ***Klupczynska Agnieszka, Dereziński Paweł, Plewa Szymon, Pawlak Magdalena, Garrett Timothy J., Rubio Vanessa Y., Kokot Zenon J., Matysiak Jan***

Honeybee venom is a natural product that pose danger to human health, but also have therapeutic properties. Peptides and proteins constitute the majority of its dry mass. However, there are also multiple low-molecular-weight compounds present in honeybee venom, which have various biological functions. The aim of the study was to identify and quantify the metabolites present in that bee product. A quadrupole-Orbitrap mass spectrometer coupled to a liquid chromatograph was used for untargeted metabolomics. Moreover, two targeted methodologies were applied that utilized a triple quadrupole mass spectrometer coupled to a liquid chromatograph. Polar metabolites (amino acids, biogenic amines, organic acids) were determined with chromatographic separation, while lipid metabolites were analyzed using flow injection analysis. The untargeted research allowed to identify several dozens of metabolites present in honeybee venom, which belong to such classes as: amino acids, catecholamines, organic acids, fatty acids, purines, pyrimidines and saccharides. 42 metabolites were quantified using targeted platforms. Among metabolites determined in the highest amount were citric acid, glycine, proline, dopamine and histamine. The research revealed that honeybee venom is a highly complex matrix not only in relation to proteins, but also to metabolites. Moreover, the variability in the composition of venom was observed. Factors influencing the changes in venom composition need to be studied and the composition need to be controlled, i.a. in order to ensure the safety of using venom as a product for medical formulations. The project received support from the National Science Centre, Poland (grant number: 2016/23/D/NZ7/03949).

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BIOMEDICAL

P-226 Peripheral vs. Cord blood: assessment of inflammatory, growth factors and metabolomic profile for the production of serum eye drops

PRESENTING AUTHOR: ***Thelma Pertinhez, University of Parma, Dept. Medicine and Surgery, Italy***

CO-AUTHORS: ***Manuela Guardi, Lucia Merolle, Chiara Marraccini, Roberto Baricchi***

Dry Eye Disease is characterized by tear increased osmolality and inflammation. Serum eye drops have emerged as a therapy for these ocular surface disorders (1). The rationale for the topical use of serum drops is based on their composition similarity to tears, due to the presence of growth factors, cytokines and vitamins, essential to maintain corneal homeostasis and wellness. Given the lack of guidelines for serum drops production, our aim was to compare the inflammatory, growth factors and metabolomic profiles from two homologous blood sources: cord blood serum (CBS) and adult peripheral blood serum (PBS), in order to pave the way for a standardization preparations procedure. This study involved S. Orsola-Malpighi Teaching Hospital (Bologna) and Azienda USL-IRCCS di Reggio Emilia. Metabolomics was performed by Nuclear Magnetic Resonance and Luminex Assay was used to measure cytokines and growth factors. Over 40 metabolites were identified and quantified. We found significant differences between the two blood-derived serum drops, with higher concentration of metabolites in CBS than PBS. The main differences were in choline, β-hydroxybutyrate, acetoacetate and glucose levels. As expected CBS showed higher concentration of TGF-α, β-NGF, FGF levels with respect to PBS. Inflammation pathway was not stimulated in CBS and nor in PBS. These results indicate that both products are safe, although the high level of TGF-α might be dangerous for patient with neoplastic disease. 1. Hessen M, Akpek EK. J Ophthalmic Vis Res. 2014, 9:240-50. Supported by Centro Regional Sangue, RER, Italy.

P-227 LC-MS/MS Method Development and Validation for Potential Early Biomarkers in Parkinson's Disease

PRESENTING AUTHOR: ***Jorge Sáiz, Universidad San Pablo CEU, Spain***

CO-AUTHORS: ***Marcela Konjevod, Alberto Bergareche, Pilar Amiano, Fernando Goñi, Eva Ardanaz, José María Huerta, Coral Barbas***

Parkinson's disease (PD) is an age-related, debilitating, severe neurodegenerative disorder characterized by degeneration of dopaminergic neurons. The identification of biomarkers for the early diagnosis of PD could improve treatment and decrease misdiagnosis. The aim of this study was to develop and validate LC-MS/MS methods that will allow to find compounds that could represent potential biomarkers. The study enrolled plasma samples as part of "The European Prospective Investigation into Cancer and Nutrition" study that were collected from healthy donors, who were followed up for 15 years. Among them, some developed PD in the following years. These samples are of great value for the discovery of biomarkers for the early diagnosis of PD and makes this study a high risk project involving the validation of biomarkers in healthy subjects. The analytes were chosen according to findings in previous studies on Parkinson's disease to be measured in target. 5 methods were developed in LC-QqQ-MS for the quantitation of groups according to their chemical characteristics. The methods were validated in terms of linearity, LOQ, repeatability, intermediate precision and stability. The LOQ for most of the compounds were below 500 ppb. The results showed good intermediate precision (1.8-32.4 %RSD), repeatability (0.3-10.2 %RSD) and stability at 4º C, as well as at room temperature for the analyzed compounds. Recoveries were found to be between 40 and 100 %. Statistically significant compounds belonged to the groups of fatty acids, sugars, alcohols and organic acids.

P-228 A Novel Metabolic Signature To Predict the Requirement of Dialysis or Renal Transplantation in Patients with Chronic Kidney Disease

PRESENTING AUTHOR: ***Helena U. Zacharias, University Medicine Greifswald, Germany***

CO-AUTHORS: ***Michael Altenbuchinger, Ulla T. Schultheiss, Claudia Samol, Fruzsina Kotsis, Inga Poguntke, Peggy Sekula, Jan Krumsiek, Anna Köttgen, Rainer Spang, Peter J. Oefner, Wolfram Gronwald***

Identification of chronic kidney disease patients at risk of progressing to end-stage renal disease (ESRD) is essential for treatment decision-making and clinical trial design. Here [1], we explored whether proton nuclear magnetic resonance (NMR) spectroscopy of blood plasma improves the currently best performing kidney failure risk equation, the so-called Tangri score. Our study cohort comprised 4640 participants from the German Chronic Kidney Disease (GCKD) study, of whom 185 (3.99%) progressed over a mean observation time of 3.70 ± 0.88 years to ESRD requiring either dialysis or transplantation. The original four-variable Tangri risk equation yielded a C statistic of 0.863 (95% CI, 0.831–0.900). Upon inclusion of NMR features by state-of-the-art machine learning methods, the C statistic improved to 0.875 (95% CI, 0.850–0.911), thereby outperforming the Tangri score in 94 out of 100 subsampling rounds. Of the 24 NMR features included in the model, creatinine, high-density lipoprotein, valine, acetyl groups of glycoproteins, and Ca2+-EDTA carried the highest weights. In conclusion, proton NMR-based plasma fingerprinting improved markedly the detection of patients at risk of developing ESRD, thus enabling enhanced patient treatment. [1] Zacharias, et al., A Novel Metabolic Signature To Predict the Requirement of Dialysis or Renal Transplantation in Patients with Chronic Kidney Disease, Journal of Proteome Research, 2019, DOI: 10.1021/acs.jproteome.8b00983.

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BIOMEDICAL

P-229 DAC-Met : Exploring the metabolic fate of the herbal components in “maoto” decoction through a differential annotation strategy

PRESENTING AUTHOR: ***Katsuya Ohbuchi, Tsumura & Co., Japan***

CO-AUTHORS: ***Nozomu Sakurai, Hiroyuki Kitagawa, Hirotaka Kushida, Akinori Nishi, Masahiro Yamamoto, Kazuhiro Hanazaki, Masanori Arita***

Natural products are attractive as drug leads. Traditional herbal medicine (THM) is expected to overcome the limitations of a single chemical agent, and is prescribed by over 80% of medical doctors in Japan together with Western drugs. To elucidate their molecular action, comprehensive profiling of the THM components and their bio-conversion is essential. We present a data-independent (non-targeted) strategy, named DAC-Met (Differential Annotation of Converted Metabolites), to explore metabolized plasma components using high-resolution mass spectrometry. As a showcase, we chose the effect of “maoto”, a mixture of ephedra herb, apricot kernel, cinnamon bark, and glycyrrhiza root, widely prescribed for febrile symptoms including fever and influenza in Asian countries. Maoto was administered to four healthy volunteers. Blood samples were collected before and after the maoto treatment, 7-time points in total. We performed plasma metabolome analyses and found maoto-derived metabolites, including known major components (ephedrine, prunasin, cinnamic acid, and glycyrrhetinic acid). Using the mass difference of major bio-conversions, we additionally estimated the structure of 11 unidentified metabolites, and 4 were later confirmed with authentic standards. With literature knowledge, we also reconstructed the metabolic pathway of maoto components. From time-resolved measurements, their kinetic profiles became clear too: many showed rapid absorption and elimination but glycyrrhetinic acid and related conjugates became prominent 4 hours after administration. The converted metabolites and their diverse kinetics revealed in this study exemplify the complex mechanism of THM action. This talk covers detailed strategy of our differential analysis and the future prospect of natural product research.

P-230 Lipidome modules underlying subclinical osteoporosis and atherosclerosis comorbidity: The Cardiovascular Risk in Young Finns Study

PRESENTING AUTHOR: ***Binisha Hamal Mishra, Tampere University, Finland***

CO-AUTHORS: ***Pashupati P. Mishra, Nina Mononen, Mika Kähönen Olli T. Raitakari, Reijo Laaksonen, Terho Lehtimäki***

Background: Evidences suggest that atherosclerosis and osteoporosis are comorbid conditions. However, the underlying mechanism is largely unknown. Subjects and methods: System-level analysis of untargeted serum lipidome was performed to identify networks of lipid species underlying atherosclerosis and osteoporosis comorbidity. The study is based on Young Finns Study cohort from 2007 follow-up (number:1194, age:30-45 years, women:59.30%). Carotid and bulbus intima media thickness was used as proxy for subclinical atherosclerosis. Total mineral density and area of heel bone, distal radius, distal radius spongy bone, radial shaft cortical bone, distal tibia, distal tibia spongy bone and tibial shaft cortical bone were used as proxy for subclinical osteoporosis. Co-expression network analysis was performed to identify modules of interconnected lipid species. Expression profiles in modules were summarized by first principal component termed as module eigenlipid. Lipid modules that were significantly correlated with both bone and artery variables were considered to be related to comorbidity. Results: A module with 124 lipid species was significantly associated with both artery (Pearson correlation: 0.16, p-value: 6e-08) and bone variables (Pearson correlation: 0.23, p-value: 1e-15). Majority of the lipid species in the module belonged to classes glycerolipid, glycerophospholipid and sphingolipid. Glycerolipids were found statistically most strongly associated with the comorbidity. The identified module contained high-risk ceramides that confirms previously suggested association between ceramides and cardiovascular outcomes, and suggests its potential role in subclinical osteoporosis. Conclusion: This study identified a lipid module associated with subclinical phase of atherosclerosis and osteoporosis comorbidity and provides potential biomarker for the comorbidity diagnosis.

P-231 LC-MS analysis of free fatty acids in acromegaly patients

PRESENTING AUTHOR: ***Bettina Gürtl, CeMM - Research Center for Molecular Medicine, Austria***

CO-AUTHORS: ***Paul Fellinger, Yvonne Winhofer-Stöckl, Kristaps Klavins***

Acromegaly is a rare disease resulting from an overproduction of growth hormone which leads to changes in the physical appearance and inner organs. Acromegaly patients also display an extremely low hepatocellular lipid content, despite pronounced insulin resistance. Therefore, studying acromegaly may help to elucidate underlying antisteatotic pathways in non-fatty liver disease. Fatty acids play an essential role in lipogenesis and hence free fatty acid levels and distributions could be used to reflect alterations in these pathways. In this work a comprehensive analysis of free fatty acids in plasma samples from acromegaly patients was performed by liquid chromatography-mass spectrometry (LC-MS). The analysis of fatty acids is often conducted by gas chromatography-mass spectrometry due to the low chromatographic performance and ionization efficiency of LC-MS. To overcome these challenges, a chemical derivatization reaction, based on 2-hydrazinoquinoline was established. The sample preparation was optimized by testing different extraction solvents, evaluating the influence of used consumables and investigating different durations of the derivatization reaction. For the LC-MS analysis a reversed phase chromatographic separation coupled to a high resolution mass spectrometer was used. The developed method provides a comprehensive coverage of 25 fatty acids including short- and long-chain fatty acids. Plasma samples from 15 patients with active acromegaly and 17 healthy controls, matched for age, BMI, gender and body composition (fat free mass), were analyzed using the established workflow. The obtained quantitative profiles of free fatty acids showed that the concentration of the majority of detected free fatty acids was lower in acromegaly patients.

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BIOMEDICAL

P-232 Fish mucus collection strategies for metabolomics: a distinct response to Aqui-S and benzocaine anaesthetics

PRESENTING AUTHOR: *Lada Ivanova, Norwegian Veterinary Institute, Norway*

CO-AUTHORS: *Rangel-Huerta, O.D., Tartor, H., Gjessing, M.C., Dahle, M.K., Thoen, E., Uhlig, S.*

Fish mucus has recently received significant interest in aquaculture. Mucus on accessible/reachable mucosal surfaces including skin and gills can be collected in a simple and non-invasive manner, providing the relevant information about the health status of the fish. However, the sampling approach may be challenging source of bias in comparative metabolomics of fish skin and gill mucus. Therefore, standardisation of sampling procedures, as well as increasing the understanding of potential sources of error are highly valuable. Different anaesthetics facilitate work with fish and reduce the level of stress, respecting the refining principle of using fish as laboratory animals. The addition of anaesthetics to the water is a common procedure prior to sampling in aquaculture. There is also a trend towards reducing the use of synthetic anaesthetics to avoid unwanted effects on the environment. Therefore, there is a growing interest in the use of natural products such as clove oil (Aqui-S) as a viable alternative to synthetic anaesthesia. This study aims to explore the effect of Aqui-S and benzocaine on the composition of the skin and gill mucus obtained after sedation of the fish. By using targeted and untargeted metabolomics, alterations in the metabolome of fish mucus induced by both anaesthetics have been evaluated. The results will be used further to improve experimental designs used in metabolomics, realising such alterations.

P-233 Evaluation of inhibitory effect of grapefruit juice on intestinal CYP3A activity

PRESENTING AUTHOR: *Yujin Lee, Seoul National University, South Korea*

CO-AUTHORS: *Soyoung Lee, Andrew HyoungJin Kim, Joo-Youn Cho*

The cytochrome P450 (CYP) 3A enzyme is major phase I drug metabolizing enzyme which accounts for 40% and 80% of total CYP in the liver and intestine, respectively. Among the factors affecting CYP3A activity, grapefruit juice is known to inhibit intestinal CYP3A activities which may lead to undesired drug interaction. We aimed to evaluate the intestinal CYP3A inhibitory effect by grapefruit juice using midazolam and endogenous metabolic markers. Fifteen Korean healthy male subjects completed the study. Subjects received oral and intravenous midazolam as probe drug. CYP3A activity was evaluated using midazolam clearance and endogenous metabolic markers. To inhibit intestinal and total (both hepatic and intestinal) CYP3A activities, grapefruit juice and clarithromycin were used, respectively. Urinary and plasma steroids were quantified by using GC-MS and the concentration of midazolam and its metabolites were quantified using LC-MS. In total CYP3A inhibition phase, urinary markers, 6β-OH-cortisone/cortisone and 6β-OH-cortisol/cortisol, and the concentration of plasma marker, 4β-OH-cholesterol, decreased 0.4, 0.5 and 0.3-fold, respectively. Midazolam clearance was also significantly decreased 0.3-fold compared to that of control. However, in intestinal CYP3A inhibition phase, both midazolam clearance and endogenous metabolic markers were not significantly decreased except for 6β-OH-cortisone/cortisone ratio was significantly decreased 0.6-fold compared to that of control. In conclusion, the inhibitory effect of clarithromycin on CYP3A activity was significant. However, the inhibitory effect of grapefruit juice was negligible. Therefore, we speculate that there will be no significant interaction between grapefruit juice and CYP3A substrate drugs.

P-234 Central and peripheral metabolomics in a model of depression: biosignatures of drug responder and non-responder mice

PRESENTING AUTHOR: *Sylvie Mavel, University of Tours, France*

CO-AUTHORS: *Sylvie Mavel, Antoine Lefèvre, Marc Legrand, Laurent Galineau, Lydie Nadal-Desbarats, Catherine Belzung, Patrick Emond*

Major depressive disorder is a heterogeneous disorder with a wide spectrum of symptoms leading to disability, suicide and physical disorders. Selective serotonin reuptake inhibitors (SSRIs) are ones of the most commonly used drugs of treatment of depression. For unknown reasons, a great number of patients do not show any improvement during drug treatment (30-40%). The underlying molecular mechanisms of depression remain unknown. To improve knowledge of pathophysiology and to search biomarkers to help prognostic of antidepressant treatment response in patient, we employed a LC-HRMS metabolomics approach in a BALB/c induced mouse model of depression to investigate metabolic changes in 6 specific brain regions, after 5 weeks with a fluoxetine treatment. From 770 cumulated targeted metabolites, 68 discriminant Variable Importance in Projection (VIP) were shown to discriminate vehicle-treated group from drug responder and non-responder mice, after OPLS-DA. This central metabolomics approach is completed by a peripheral analysis through plasma analysis. A less robust OPS-DA model was obtained, but 27 discriminant metabolites (over 197 robustly analyzed) were altered in plasma. Comparison of central VIP with peripheral VIP leads to 7 common metabolites. This study shows the complementary of these two compartments, i.e. cerebral and plasma metabolome. From this, specific brain regions shown particular metabolism which could explain some aspects of the depressive pathophysiology and some biomarkers could be proposed to anticipate antidepressant treatment response.

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BIOMEDICAL

P-235 Differences in metabolic profiles of venous and arterial umbilical cord blood

PRESENTING AUTHOR: ***Olle Hartvigsson, Chalmers University of Technology, Sweden***

CO-AUTHORS: ***Alastair Ross, Carl Brunius, Malin Barman, Ann-Sofie Sandberg***

Analysing umbilical cord blood is important for assessing neonatal health and development. The easiest and most common collection procedure is to squeeze blood out of the cord after it is severed. Hence a mixture of venous (from mother to child) and arterial (from child to mother) blood is collected. This study aimed to determine key differences in metabolite profiles between venous and arterial cord blood plasma. The metabolome of venous, arterial and mixed squeezed umbilical cord blood was analysed from 52 children using a combination of targeted and untargeted GC-MS/MS. Data was analysed by multilevel Random Forest (ML-RF) in a repeated double cross validation framework incorporated with unbiased variable selection. In pairwise analysis of arterial and venous blood, approximately 75% of samples were correctly classified (p=0.0078). Arterial blood had higher concentrations of glucose, sorbose and galactose than venous blood which contained higher levels of α-ketoglutaric acid, L-glutamic acid, homocysteine and several other metabolites. Mixed blood had a metabolic profile that was in-between the arterial and venous blood, but could not be classified properly by multivariate models. Our results highlight that cord blood sampling with non-systematic mixing of arterial and venous blood induces undesirable variability in metabolomics analyses. We therefore conclude that control of the sampling procedure is imperative during metabolomics analyses, especially when energy or amino acid metabolism are relevant for the research question.

P-236 Comparison of plasma metabolic profiling in allergic rhinitis and asthma by 1H NMR in early childhood

PRESENTING AUTHOR: ***Meng-Han Chiang, Research Fellow, Taiwan***

CO-AUTHORS: ***Chih-Yung Chiu, Gigin Lin***

Allergic rhinitis and asthma are both chronic heterogeneous disorders with the high prevalence of coexistence. Clinically, house dust mite sensitization with elevated serum immunoglobulin E (IgE) levels is a significant factor in relation to both allergic rhinitis and asthma. However, few studies have addressed the potential molecular mechanisms between allergic rhinitis and asthma, especially in children. Metabolomics provides a complementary functional approach to the molecular functions interacted with the host, offering the opportunity to characterize diseases effectively. The aim of this study was to investigate plasma metabolic profiling in children with allergic rhinitis and asthma. Plasma samples were collected and metabolites were analyzed by 1H-nuclear magnetic resonance spectroscopy (NMR). NMR spectra were processed by an open source software NMRProcFlow and subsequently analyzed by MetaboAnalyst. In this study, a total of 111 children with mean age of 4.9 ± 0.5 years were enrolled. Among them, there were 27 asthmatics, 44 allergic rhinitis, and 40 healthy controls. After analysis, total 104 buckets refer to 50 known metabolites were identified by Chenomx software. Compared to healthy controls, six and five metabolites were significantly different in children with allergic rhinitis and asthma respectively. Among them, allergic rhinitis associated hypoxanthine, glutamate, and phenylalanine were positively changed with mite-allergen specific IgE levels, whereas asthma associated glutamine and arabinose were inversely changed in opposing directions. Clinically, a comprehensive analysis of functions studies related to these 8 metabolites could potentially provide further prevention and treatment in children with allergic rhinitis and asthma.

P-237 Endometrial Receptivity in Women with Dormant Genital Tuberculosis: A Molecular and Metabolomic Approach

PRESENTING AUTHOR: ***Koel Chaudhury, School of Medical Science and Technology, Indian Institute of Technology Kharagpur, India***

CO-AUTHORS: ***Elavarasan Subramani, Baidyanath Chakravarty***

Genital tuberculosis (GTB) in women is one of the common causes of infertility in emerging countries. The important problem in diagnosing and treating GTB is that it often exists in a dormant form without any clinical symptoms. There is a general consensus that GTB affects implantation due to ovarian and endometrial involvement. Behavior of the endometrium during the window of implantation in asymptomatic infertile women having dormant GTB is not well understood. The possible molecular association between dormant GTB and endometrial receptivity is, therefore, explored. Reduced levels of endometrial receptivity markers suggest impairment in the receptive status of the endometrium in dormant GTB women. Also, alterations in endometrial thickness, blood flow parameters and vascular endothelial growth factor indicate poor angiogenesis during implantation window. Compromised LIF-STAT3 signaling pathway could possibly be responsible for repeated implantation failure. Metabolomic studies indicate a clear metabolic differentiation in the metabolic profile of endometrium and serum between women with dormant GTB and controls. The significantly altered endometrial tissue and serum metabolites could be largely related to energy metabolism and amino acid biosynthesis. This underlines the metabolic influence of tubercle infection, even in its dormant form. Expression of several serum metabolites followed a similar pattern as that observed in the endometrium of dormant GTB women. The potential of these metabolites as putative diagnostic markers of dormant GTB seems promising.

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***AWARD WINNERS**

BIOMEDICAL

P-238 Study of fetal pig maturity in relation with neonatal survival using a multi-fluids metabolomic approach

PRESENTING AUTHOR: ***Laurence Liaubet, INRA-GenPhySE, France***

CO-AUTHORS: ***Gaëlle Lefort, Nathalie Vialaneix, Hélène Quesnel, Marie-Christine Père, Yvon Billon, Laurianne Canario, Nathalie Iannuccelli, Cécile Canlet, Alain Paris, Rémi Servien, Laurence Liaubet***

Selection for prolificacy and lean growth in swine has been associated with a substantial increase in piglet mortality. The first 24-48 hours after birth represent the most critical period. A major determinant for early survival is piglet maturity at birth, which strongly relies on the process of maturation during the last month of gestation. The objective of the current study was to compare progeny from Large White and Meishan pigs, which differ for piglet survival and vitality, to describe the metabolic status of the piglets. Thirty-nine sows were anesthetized at 90 or 110 days after conception (average gestation term: 114 days). Their fetuses (on average 15.7 per sow) were quickly obtained by caesarean section and this study focuses on the plasmatic, the urinary and the amniotic liquid metabolomes (1H-NMR) of 507 to 604 fetuses. Raw 1D Bruker spectral data files were treated with ASICS, an R package available on Bioconductor. ASICS allowed the direct identification and quantification of about 90 metabolites from a library of 190 metabolites, with about 60 metabolites per fluid, among which 39 were in common in the three fluids. A multivariate analysis and a mixed model were applied to the quantification results to explain the differences between the gestational stages and the breeds. The results provide new insights in the biological pathways involved in piglet maturation. Grants: ANR ANR-09-GENM005 (PORCINET) and ANR-16-CONV-0004 (#DigitAg), INRA (divisions GA, SA, MIA).

P-239 Neonatal hair profiling of monochorionic twins can be implicated to reflect a longitudinal metabolic phenotype of compromised fetal growth rate and abnormal umbilical artery flow

PRESENTING AUTHOR: ***Ting-Li Han, First affiliated Hopstial of Chonqing Medical University, China***

CO-AUTHORS: ***Jing Yang, Yuan Wei, Yang Yang, Zhang Hua, Yangyu Zhao***

Selective intrauterine fetal growth restriction (sIUGR) in monochronic diamniotic twins, especially types 2&3 with abnormal umbilical artery Doppler, results in increased risks of fetal/perinatal mortality and postnatal disability. Hair metabolome profiles of neonates were characterized to reflect the long-term pathophysiological changes across different clinical forms of sIUGR twins. Hair samples were collected at delivery from 10 pairs of type 1 sIUGR twins, 8 pairs of types 2&3 sIUGR twins, and 11 pairs of uncomplicated twins. The hair metabolome was characterized by gas chromatography-mass spectrometry. PLS-DA analysis and ROC curves were applied to screen for metabolites between/within twin pairs in different sIUGR and uncomplicated twin subgroups. A generalized estimating equation (GEE) model was implemented to correlate hair metabolites to birthweight and fetal growth rate within/between sIUGR co-twins. Metabolic pathway analysis and metabolic network reconstructions were performed using the KEGG database. Our results demonstrated that the hair metabolite profiles of different sIUGR subclinical forms were associated with the averaged fetal growth rate after 28 weeks of gestation but not birthweight. The hair profiles were capable of discriminating type2&3 sIUGR twins from uncomplicated twins. In particular, the metabolites 2-aminobutyric acid, cysteine, alanine, proline, valine, and tyrosine areas under the ROC curve were above 0.9. The metabolic pathway analysis highlighted the associations of sIUGR twins with abnormal umbilical artery flow including nutrient depletion pathway, glutathione metabolism, and attenuated nerve development. This study offers novel insight into evaluating the severity of intrauterine ischemia and hypoxia for T2&3 sIUGR twins, through the neonatal hair metabolome.

P-240 Early phenotypic alterations of newborns suffering from Hypoxic-Ischemic Encephalopathy with pathologic cerebral magnetic resonance imaging

PRESENTING AUTHOR: ***Julia Kuligowski, Health Research Institute La Fe, Spain***

CO-AUTHORS: ***José David Piñeiro-Ramos, Antonio Núñez-Ramiro, Anna Parra-Llorca, Juan Martínez-Rodilla, Roberto Llorens, Ángel Sánchez-Illana, Guillermo Quintás, Máximo Vento***

Hypoxic-ischemic encephalopathy (HIE) the one of the major causes of neurodevelopmental impairment in the pediatric age. Therapeutic hypothermia (TH) initiated within 6 h of birth is now established as the standard of care for infants with moderate and severe HIE. TH has significantly reduced mortality and improved neurological outcome in survivors. However, no objective bedside test is available for an accurate and early diagnosis of HIE. This study elucidates early metabolic changes in newborns with HIE in order to select potential disease specific target biomarkers capable of predicting long-term adverse outcomes. We analyzed changes in the plasma metabolome employing gas-chromatography-mass spectrometry (GC-MS) and liquid chromatography-time-of-flight MS (LC-TOFMS) in a cohort of 55 asphyxiated infants who evolved to moderate/severe HIE. Hypoxia-ischemia induced brain injury was assessed employing magnetic resonance imaging (MRI) between days 4 and 8 post-partum. This study describes for the first time the evolution of the plasma metabolome of newborn infants with HIE covering the time period between birth and completion of TH. Results obtained allowed a critical assessment of the usefulness of lactate, pyruvate, and pyruvate/lactate for early outcome prediction. Besides, metabolomic profiling identified a dynamic perturbation of eleven metabolic pathways, including amino acid and purine metabolism, and the steroid hormone biosynthesis, in newborns with pathologic MRI outcomes. Future work will focus on the specific study of the steroid hormone biosynthesis pathway, which remained altered during the whole study period, to explore its potentials for biomarker discovery or adjuvant therapies to be combined with TH.

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*AWARD WINNERS

BIOMEDICAL

P-241 Evaluation of Lipid Profile Differences between Neonatal and Adult Serum

PRESENTING AUTHOR: ***Richard Beger, NCTR, United States***

CO-AUTHORS: ***Mustafa Akkoyunlu, Jiyeon Yang, Jinchun Sun***

Introduction: While lipid profiles in terms of lipid classes including total cholesterol, triglycerides, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol have been measured in newborn infants, little work has been conducted to show the lipid levels as lipid species in neonatal serum. Methods: Adult pooled sera (AS) were collected from 2 adult mice (8 to 12-week-old) for each replicate (n=5 replicates). Neonatal pooled sera (NS) were collected from 8-10 mice (1-week-old) for each replicate (n=5). An aliquot of 50 µL of the reconstituted sample was directly infused into a QTRAP 5500 MS with SelexION™ technology at a flow rate of 7 µL/min for targeted lipid profiling. Results: Lipidomics analysis results showed that total lipid amount (13 classes of lipids including neutral and polar lipids) present in NS was lower vs AS or in HINS vs HIAS, which is consistent with previous studies on newborns. Lipid classes of CE, DAG, LPC, LPE and TAG were significantly lower in NS vs AS; while lipid classes of PC, PE and SM were significantly higher in NS vs AS. The top fatty acid chain lipids differentiated NS from AS were the most abundant TAGs including (FA 16:0), (FA 16:1), (FA 18:1) and (FA 18:2). Interestingly, NS and HINS serum had higher levels of all lipid classes containing arachidonic acid (signaling molecule and a key inflammatory intermediate) chain vs AS and HIAS. Novel Aspects: Lipidomics analysis was conducted to evaluate the lipidome differences between neonatal and adult mouse sera at lipid species level.

P-242 LC-MS Based Targeted and Untargeted Metabolomics for the Identification of Potential Biomarkers in Plasma From Pediatrics With Chronic Kidney Disease

PRESENTING AUTHOR: ***Sandra Benito, University of the Basque Country, Spain***

CO-AUTHORS: ***Nora Unceta, Alicia Sánchez-Ortega, M. Aránzazu Goicolea, Ramón J. Barrio***

The assessment of chronic kidney disease (CKD) is carried out using glomerular filtration rate (GFR), which is calculated from serum creatinine concentration. However, serum creatinine concentration changes according to several factors, thus endangering CKD diagnosis, especially in early stages of the disease. In addition, pediatric CKD is related to malnutrition, development, metabolic and cardiovascular complications, leading to a 30 times higher mortality rate in paediatrics suffering from advanced CKD in comparison with their healthy counterparts. Regarding that the discovery of new biomarkers might allow earlier diagnosis as well as a better response, comprehensive research has been performed in plasma samples from paediatrics with CKD. Following targeted and untargeted metabolomics approaches 7 potential biomarkers have been found which could be useful for pediatric CKD by means of comparison of metabolic profiles: citrulline, S-adenosylmethionine, symmetric dimethylarginine, cis-4-decenoylcarnitine, n-butyrylcarnitine, bilirubin and sphingosine-1-phosphate. The use of these metabolites in addition to creatinine enables better differentiation of paediatrics with CKD and control paediatrics in comparison with the use of creatinine alone. Afterwards, these 7 biomarkers in addition to the classical biomarker creatinine have been gathered together into a routine analytical method. Aimed at evaluating the usefulness of this routine analysis method, a CKD pediatric population have been analyzed as well as a healthy pediatric population, being able to differentiate between them by means of data analysis.

P-243 Correlating plasma and liver metabolic profiles: an application to the study of organ maturation

PRESENTING AUTHOR: ***Oskar González, University of the Basque Country, Spain***

CO-AUTHORS: ***Oihane E. Albóniga, Rosa M. Alonso, Yun Xu, Royston Goodacre***

Liver plays a main role in drug metabolism, so studying the grade of maturation of this organ would help to develop more appropriate dosing regimens for paediatric population. Nevertheless, considering the invasive nature of liver analyses there are obvious ethical boundaries. In this work, we investigated the suitability of plasma as an alternative matrix to evaluate the biological age of liver. With this aim, we studied the correlation of plasma and liver metabolomic profiles obtained by UHPLC-TOF-MS for piglets of different ages. By means of Pearson correlation analysis we observed that 360 and 1784 pairs of features were significantly correlated in positive and negative ionization mode, respectively. Procrustes transformation was applied in order to assess the similarity of the clustering resulting from the data obtained from the two matrices. The dissimilarity values were low both for ESI+ (0.3753) and for ESI- (0.3673) and, hence, liver and plasma are expected to provide similar discriminatory information. Furthermore, Multiblock Principal Component analysis demonstrated to be a very suitable tool to combine the data obtained from both matrices and to better understand the clustering according to three groups: newborns, neonates and infant piglets. Considering all these results, plasma proved to be a matrix of great interest to provide insight into the maturation grade of liver. This could lead to less invasive procedures to study drug metabolism and it could be an important step forward towards an accurate dosing in paediatric population.

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*AWARD WINNERS

BIOMEDICAL

P-244 LC-MS based metabolomics: Biomarkers of organ maturation in paediatrics

PRESENTING AUTHOR: ***Oihane Elena Albóniga, Analytical Chemistry Department, Faculty of Science and Technology, University of the Basque Country, Spain***

CO-AUTHORS: ***Oskar González, María E. Blanco, Rosa M. Alonso, Yun Xu, Royston Goodacre***

Knowledge about drug metabolism in paediatric population is limited mainly due to ethical issues, scarce number of clinical trials and reduced sample size of the few paediatric studies available. In consequence, drug administration in children is often based on algorithms from allometric models optimised for adults, without considering the maturation state of key organs, such as liver, which during childhood are still in their development process. The invasiveness of liver sample collection requires the use of animal models, such as piglets, to study the paediatric population. In this study, metabolomic profiles of liver tissue, obtained from 36 piglets of different ages, were analysed by an HPLC-Q-TOF-MS system in positive and negative electrospray ionization (ESI) modes. The multivariate analysis by Principal Component Analysis (PCA) showed a perfect clustering of the three groups of piglets (newborns, neonates and infants). By univariate analysis, 91 features (m/z-tr) at ESI+ and 48 features at ESI- differentiating the three groups of piglets were found. The HPLC-MS/MS analysis of these significant features and their comparison with Metlin, HMDB, mzCloud and MyCompoundID databases allowed the identification of 13 acylcarnitines, such as acetylcarnitine, butyryl-L-carnitine, 2-methylbutyroyl-carnitine or palmitoyl-L-carnitine, among others, suggesting the involvement of the fatty acids metabolism in the liver maturation state. These results provided an important contribution in paediatrics and demonstrated that metabolomics is a useful tool in this area.

P-245 Metabolomic prediction of adverse pregnancy outcomes

PRESENTING AUTHOR: ***Nancy McBride, MRC IEU, United Kingdom***

CO-AUTHORS: ***Paul Yousefi, Matthew Suderman, Caroline Relton, Deborah Lawlor***

Pregnancy-related disorders, such as gestational diabetes (GD), hypertensive disorders of pregnancy (HDP), small for gestational age (SGA), large for gestational age (LGA) and preterm birth (PTB), can have long-lasting adverse effects on maternal and offspring health and are a significant public health concern. Current risk factors rely on clinical predictors such as smoking, age, parity and body mass index (BMI). However, these are poor predictors. Metabolites may reflect dynamic changes in metabolic pathways of women with pregnancy-related disorders. We aim to see whether we can use metabolomics to improve prediction of adverse pregnancy outcomes. We used the Born in Bradford multi-ethnic cohort and the UK Pregnancies Better Eating and Activity Trial randomised control trial of obese pregnant women. Both have nuclear magnetic resonance (NMR) metabolite data from pregnant women at 24-28 weeks gestation. We used 159 NMR-derived metabolite measures and information on clinical predictors from 9,312 women to generate prediction models. Penalised regression was used to create predictive models with an elastic net penalty for HDP, GD, SGA and LGA from 90% of the cohort. Out-of-sample performance was conducted in a randomly allocated subset of 10% of observations that were withheld during training using receiver operating characteristic curves. We found that a model combining the metabolites and existing clinical predictors had very good discrimination for GD (AUC 0.85), good for HDP (0.76) and SGA (0.73), poorer for LGA (AUC 0.69). We will now compare this to the predictive purpose of the mass spectrometry (MS) metabolite platform.

P-246 Pre-symptomatic biomarker for preeclampsia in mid-trimester

PRESENTING AUTHOR: ***Yujin Kang, Kookmin University, South Korea***

CO-AUTHORS: ***Seung Mi Lee, Joong Shin Park, Do Yup Lee***

Preeclampsia (PE) is a hypertensive disease associated with pregnancy that has not yet been clearly elucidated. The disorder can affect fetus and pregnant women, which in turn necessitates special care. In particular, it may cause complications such as fetal growth retardation, premature birth, renal dysfunction, and chronic hypertension in pregnant women. Sometimes, preeclampsia is very similar to symptoms of normal pregnancy such as weight gain, swelling and headache and some mothers have no symptoms at all. Accordingly, it is very important to properly diagnose preeclampsia through periodic antenatal screening and early diagnosis. In our current study, metabolic profiling of maternal plasma samples in mid-trimester was performed using gas chromatography-time of flight mass spectrometry (GC-TOF MS) and liquid chromatography orbitrap mass spectrometry (LC-Orbitrap MS). A total of 330 metabolites were semi-quantitatively identified. Primarily, partial least squares-discriminant analysis (PLS-DA) demonstrated a clear discrimination between pregnant women with preeclampsia and healthy controls. Subsequent binary logistic regression analysis constructed a potential biomarker recomposite with 3 metabolites. The predictive model successfully discriminated preeclampsia group in the mid-trimester (AUC = 0.801).

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BIOMEDICAL

P-247 Investigation of Early Diagnosis of Neonatal Sepsis by Untargeted Metabolomics Approach

PRESENTING AUTHOR: ***Giuseppe Giordano, Padova University, Women's and Children's Health Department, Italy***

CO-AUTHORS: ***Veronica Mardegan, Antonella Gucciardi, Matteo Stocchero, Paola Pirillo, Gabriele Poloniato, Mauro Naturale, Eugenio Baraldi.***

Neonatal sepsis is a complex infection-induced systemic inflammatory response syndrome and it is a main cause of mortality and neurologic sequelae in newborns. An early and accurate detection of sepsis is mandatory in neonates, since the clinical course of the infectious process can be fulminant, leading to septic shock and death within hours after the first clinical symptoms. To date, there is still no reliable biochemical marker of neonatal sepsis. The aim was to compare the metabolic profile of urine collected within 24 hours of birth between preterm neonates affected by early onset sepsis (EOS) and healthy preterm infants, searching for a specific metabolic profile enabling the identification of preterm newborns prone to develop EOS. Infants who developed a septic episode within 72 hours of birth were enrolled as cases (9 cases and 10 controls who did not developed a septic episode). The urine samples underwent untargeted metabolomic analysis using LC-MS. The data were analyzed by multivariate and univariate data analysis tools. For each data set, a multivariate biomarker showing area under the ROC curve calculated by cross-validation greater than 0.80 was built. The annotated variables were submitted to over-representation pathway analysis that highlighted the perturbation of 6 metabolic pathways. Neonates with EOS showed a specific metabolic profile compared to those of newborns not affected by sepsis at the onset of infection. Results of this research support the effectiveness of metabolomics in exploring biochemical pathways of neonatal sepsis, potentially providing novel putative biomarkers for early diagnosis.

P-248 Metabolomic barometer of gestational and postpartum weight in overweight pregnant women

PRESENTING AUTHOR: ***Chung-Ho Lau, Imperial College London, United Kingdom***

CO-AUTHORS: ***Victoria Taylor-Bateman, Panagiotis A Vorkas, Gonçalo Graça, Elena Chekmeneva, Timothy M.D. Ebbels, Linda Van Horn, Queenie Chan, Elaine Holmes***

Background: Obesity amongst women of reproductive age is increasingly common in developed nations and has been shown to adversely affect childhood cardio-metabolic, respiratory and cognitive-related health outcomes in offspring. Metabolomic signatures of obesity are readily captured in biofluids samples and could potentially provide a molecular barometer for monitoring excessive gestational weight gain (GWG) and postpartum weight loss (WL) in overweight/obese pregnant women. Methods: Urine and blood plasma samples were collected from 114 overweight or obese ethnically diverse pregnant women from Chicago (USA), as part of a randomised diet and lifestyle intervention trial (Maternal Offspring Metabolics Family Intervention Trial; www.clinicaltrials.gov NCT01631747). Blood plasma lipids and urine samples at 15 weeks, 35 weeks of gestation, and at 1 year postpartum were respectively analysed by LC-MS and NMR. Results: Urinary 4-deoxyerythronic acid was found positively correlated to body mass index (BMI) and a broad spectrum of alterations in levels of blood plasma phosphatidylcholines, lysophospholipids, and sphingomyelins were associated with BMI, GWG, and WL. Specifically, several plasmanyl-/plasmenyl-phospholipids were negatively associated with GWG, and lysophosphatidylcholines (including LPC 20:4) were positively associated with WL. Multiple lipids with apparent 18:2 fatty-acid chains were significantly associated with GWG/ WL, suggesting linoleic acid, an essential nutrient, may play an important role in prenatal and postpartum weight management. Conclusions: Maternal obesity-related parameters are associated with urine and plasma metabolomic profiles, which could be further exploited to evaluate the beneficial effect of diet and lifestyle intervention in pregnancy.

P-249 A population-based resource for intergenerational metabolomics analyses in pregnant women and their children: the Generation R Study

PRESENTING AUTHOR: ***Ellis Voerman, Erasmus University Medical Center, Netherlands***

CO-AUTHORS: ***Vincent W.V. Jaddoe, Romy Gaillard***

Adverse exposures during fetal and early postnatal life may predispose children to cardio-metabolic disease in later life. Early-life developmental adaptations of metabolic pathways may underlie these associations. Metabolomics may serve a tool to disentangle these potential metabolic adaptations. In a prospective population-based cohort study among pregnant women and their children from Rotterdam, the Netherlands, we used a targeted metabolomics approach to measure metabolite concentrations in maternal blood in early-pregnancy (n=825), cord blood at birth (n=932), and child's blood at age 10 (n=508). We used liquid chromatography mass spectrometry (LC-MS/MS) to measure blood concentrations of amino acids, acyl-carnitines, polar lipids (including diacyl-phosphatidylcholines, acyl-alkyl-phosphatidylcholines, sphingomyelines, acyl-lysophosphatidylcholines and alkyl-lysophosphatidylcholines), and non-esterified fatty acids. Preliminary exploratory analyses showed weak correlations between maternal concentrations of individual metabolites and concentrations of these metabolites in their children at birth (highest Spearman's Rho: 0.36) and at the age of 10 years (highest Spearman's Rho: 0.24), respectively. Child's metabolite concentrations at birth and at 10 years of age were also weakly correlated (highest Spearman's Rho: 0.36). Whether individual metabolite concentrations or metabolite patterns are related to maternal and child adverse exposures and health outcomes is subject of future analyses. These data are an important population-based resource for metabolomics analyses in pregnant women and their children to address the early origins of health and disease.

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BIOMEDICAL

P-250 Longitudinal metabolite fluctuations in urine reveal individual variations and responses to rhinovirus challenge (BIOFLUC study)

PRESENTING AUTHOR: ***Romanas Chaleckis, Gunma University Initiative for Advanced Research (GIAR), Japan***

CO-AUTHORS: ***Anirban Sinha, Isabel Meister, Pei Zhang, Craig Wheelock, Rene Lutter, Peter J. Sterk***

Asthma is one of the major chronic noncommunicable diseases affecting over 300 million people worldwide. Exacerbations are the major burden in asthma management which are primarily triggered by rhinovirus exposures. Temporal metabolite fluctuations in biofluids could be used to monitor and predict the disease exacerbations. However, little is known on the individual variation of the metabolome, especially on the scale of weeks-to-months which is important to monitor health trajectories. Here we present the temporal dynamics of urinary metabolites from BIOFLUC study (Netherlands trial register: NL5317) in healthy and asthmatic subjects before and after a rhinovirus challenge. This a prospective longitudinal follow-up study in 12 healthy and 12 asthmatics over a period of 3 months with an experimental rhinovirus intervention after 2 months. Multiple samples were collected from different sources 3 times a week amounting to more than 35 samples per subject. Urine LC-MS metabolomic profiling of polar metabolites on zic-HILIC column using all ion fragmentation data acquisition was performed. Data was processed with MS-DIAL software and metabolite identities confirmed by accurate mass, retention time and MS spectra. Metabolite clustering by the CVs (coefficients of correlation) revealed several groups, with amino acid metabolites being less and food/drug metabolites being most variable. Individual responses were observed after rhinovirus challenge with spikes of cytosine levels in many, but not all study participants. Urinary metabolomics provides an accessible avenue to characterize individual baseline urine metabolites in healthy and asthmatic subjects thereby helping to identify compounds associated with disease presentation.

P-251 Urinary metabolomics identifies molecular signatures associated with bronchopulmonary dysplasia (BPD) and birth-term

PRESENTING AUTHOR: ***Craig Wheelock, Karolinska Institute, Sweden***

CO-AUTHORS: ***Isabel Meister, Romanas Chaleckis, Pei Zhang, Takashi Izumi, Marika Ström, Petra Um-Bergström, Eva Berggren Broström, Erik Melén, Magnus Sköld, Åsa M. Wheelock***

Chronic obstructive pulmonary disease (COPD) is a rising global health problem, currently affecting >10% of the global population. While smoking is the major risk factor for developing COPD, 20% of COPD patients have never smoked. Prematurely born, particularly those who developed bronchopulmonary dysplasia (BPD) in the neonatal period, are a growing group of subjects at-risk of developing early-onset COPD. BPD affects 10-30% of premature children, corresponding to 1% of all newborns. We present here the urine metabolomics of young adults from the LUNAPRE cohort (n=96; www.clinicaltrials.gov/ct2/show/NCT02923648), consisting of 4 non-smoking groups, age 18-23: i) prematurely born (<32 weeks of gestation) BPD survivors (n=26); ii) healthy born preterm (<32 weeks, n=23), iii) healthy born at term (>37 weeks, n=24), and iv) mild asthmatics born at term (>37 weeks, n=23). We performed LC-HRMS-based urinary metabolomics in combination with targeted quantification of the urinary eicosanoid profiles. Metabolomics identified 68 AMRT-confirmed metabolites, of which 39 were also MS/MS confirmed. We observed a 2.4-fold increase in carnitine levels and a 0.6-fold decrease in acetyl-glucosamine levels in the pre-term BPD survivors compared to healthy pre-term group. Multivariate analysis found that urinary eicosanoid profiles differed between the BPD survivor group relative to both the healthy pre-term group (p=0.007) and the asthma group (p=0.02). The separation with both groups was primarily driven by downregulation of the thromboxane and prostaglandin D2 pathways. These preliminary analyses suggest that molecular signatures associated with BPD and birth-term persist into adulthood and may be useful for understanding this unique sub-group of COPD patients.

P-252 High power vaping modifies cardiorespiratory parameters and urine metabolome: a randomized crossover trial in heavy ecigarette users

PRESENTING AUTHOR: ***Vanessa Tagliatti, University of Mons, Belgium***

CO-AUTHORS: ***Martin Chaumont***

The use of electronic cigarettes has increased since its marketing. However, health effects remain largely unknown due to its newness. In the present study, effect of propylene-glycol and glycerol, e-cigarette constituents that facilitate liquid vaporization and nicotine transport, is evaluated in short-term cessation of e-cigarette vaping in regular-users. Subjects were enrolled in a randomized investigator-blinded crossover-three-period study for five days before the experimental session (nicotine e-cigarettes: nicotine-session; nicotine-free vaping: nicotine-free-session; complete cessation of e-cigarette vaping: stop-session). Metabolomic analysis, by proton nuclear magnetic resonance, was performed on urine samples and baseline clinical/biological cardiorespiratory parameters were assessed at the beginning of each experimental session. In comparison to nicotine- and nicotine-free-session, stop-session was characterized by a specific urine metabolomic signature (increase in hippurate and decrease in hydroxyisovalerate). This modified baseline urine metabolome sustained by an increase of serum CC16 concentration suggesting an improvement in lung inflammatory profile.

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***AWARD WINNERS**

BIOMEDICAL

P-253 1H NMR metabolomics of serum and EBC for understanding the pathophysiology of COPD associated PH

PRESENTING AUTHOR: ***Priyanka Choudhury, School of Medical Science and Technology, Indian Institute of Technology Kharagpur, India***
CO-AUTHORS: ***Nilanjana Ghosh, Mamata Joshi, Parthasarathi Bhattacharyya, Sushmita Roy Chowdhury, Koel Chaudhury***

Group III pulmonary hypertension (PH), considered to be a delayed complication of hypoxic lung diseases has a major burden of chronic obstructive pulmonary disease (COPD). COPD associated PH (COPD-PH) often gets overshadowed by the symptoms of COPD and is associated with shorter survival and worse clinical outcomes. Understanding of the etiopathogenic mechanisms responsible for PH development in COPD remains incomplete. In this study, we hypothesize that 1H nuclear magnetic resonance (NMR) based metabolomics will provide an insight into the pathophysiology of COPD-PH and will also be able to differentiate it from COPD alone at a metabolic level. Two biofluids serum and exhaled breath condensate (EBC) are explored in the present study. Paired serum and EBC samples were collected from patients with pure COPD (n=47), COPD-PH (n=31) and healthy controls (n=39). NMR spectra of the samples were acquired using 800 MHz BrukerAvance III spectrometer equipped with a cryoprobe and the data subjected to univariate and multivariate analysis. On comparing COPD-PH with COPD and controls, distinct metabolic differentiation was observed between the groups. The OPLS-DA models generated for COPD-PH vs. COPD showed good R2 and Q2 values [(a) Serum: R2Y (0.902) and Q2 (0.755) (b) EBC: R2Y (0.901) and Q2 (0.715)] indicating the robustness of the model. The identified metabolites were found to be majorly associated with glycolysis pathway, amino acid and lipid metabolism. The distinct metabolic signatures suggest that the two study groups are distinguishable from each other and also give us a preliminary idea about the pathophysiology of the disease.

P-254 Lipidomic Quantitation of Respiratory Disease: A Rapid and Comprehensive HILIC-Based Targeted Approach

PRESENTING AUTHOR: ***Giorgis Isaac, Waters Corporation, United States***
CO-AUTHORS: ***Nyasha Munjoma, Lee A. Gethings, Robert S. Plumb***

Respiratory linked conditions associated with chronic obstructive pulmonary disease (COPD), asthma, and infection are increasing. The analyses of plasma samples from three biological states of varying phenotype (control, COPD and asthma patients) were conducted. Sample extraction was performed using a simple protein precipitation with a pre-cooled isopropanol. Lipid analysis was performed using a fast (<8min), comprehensive and high-throughput targeted method which is based on UPLC-HILIC lipid class separation followed by MRM quantitation of individual lipid species. It is integrated workflow for accurate and robust measurement of a carefully selected more than 500 lipid species. Data were processed using TargetLynx and Skyline. The biological samples were randomised and two technical replicates per sample were acquired. Statistical analysis of the data revealed clear separation between the various cohorts. Unsupervised PCA resulted in the separation of healthy controls, COPD and asthma patients. Application of the metadata also revealed significant differences between smoking status, with subsets readily observed within the COPD population. Loadings plot analysis revealed that FFA, LPC, PC and SM lipid classes to be the main contributors to sample type clustering. Additional ANOVA/t-test and hierarchical clustering showed all the lipid classes referenced to be up-regulated except for PC's. A decrease in the level of PC's was observed as significant for subjects associated with smoking. Overall, PCs are a potential marker for oxidative stress (immune activation). Pathway analysis revealed several components related to inflammation, oxidative and immunity processes were identified as significant and associated with signalling, metabolic and regulatory pathways.

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***AWARD WINNERS**

NEW FRONTIERS

P-255 Integrating expression data into metabolome-wide GWAS suggests candidate genes modulating metabolite concentration

PRESENTING AUTHOR: ***Reyhan Sonmez Flitman, University of Lausanne, Switzerland***

CO-AUTHORS: ***Rico Rueedi, Sven Bergmann***

Metabolome-wide GWAS (mGWAS) search for associations between metabolites and common genetic variants within large collections of samples. NMR spectral intensities reflect metabolite concentrations, which in targeted NMR are combined to quantify a limited set of metabolites. In order to avoid the often imperfect identification and quantification of metabolites, we designed a different type of mGWAS called untargeted mGWAS, that directly tests metabolome features for association with genetic variants, not discarding any data that may have eluded identification. The effect of a genetic variant on the concentration of a metabolite tends to translate, in an untargeted mGWAS, to associations with all or some of the features corresponding to the pure NMR spectrum of the metabolite. The association profile can therefore allow for identification of the underlying metabolite using a method we call metabomatching (Rueedi et al., PLoS Genetics 2014). Here, we apply metabomatching to associations with gene expression rather than genotypic variation. To this end we used RNAseq profiles of lymphoblastoid cell lines derived from 555 CoLaus subjects for which we had also urine NMR data. Specifically, we investigated the genes whose expression levels showed strong association with metabolome features. We found that ALMS1 gene expression is strongly associated to the concentration of N-Acetyl L-Aspartate (NAA) making ALMS1 the most likely candidate gene for modulating this metabolite. In summary our study provides evidence that the integration of metabolomics with gene expression data can support mQTL analysis, helping to identify the most likely gene involved in the modulation of the metabolite concentration.

P-256 The application of artificial neural networks and deep learning to metabolomics

PRESENTING AUTHOR: ***Kevin Mendez, Edith Cowan University, Australia***

CO-AUTHORS: ***Stacey Reinke, Leighton Pritchard, David Broadhurst***

Metabolomics data are highly complex, both in terms of the number of variables and the high degree of multicollinearity between variables. As such, multivariate machine learning methods that project the data into a smaller linear “latent” space before classification are routinely used, with Partial Least Squares Discriminant Analysis (PLS-DA) being by far the most popular approach. However, it is generally observed that biological processes are nonlinear. As such, it is fair to hypothesise that linear projection techniques may not be optimal. Artificial Neural Networks (ANNs) are a class of nonlinear models introduced into the metabolomics community over 20 years ago, which failed to gain momentum due to computational requirements for optimisation, and the difficulty of interpretation. Here we present a comparison of the standard back-propagation ANN against PLS-DA and other popular machine learning algorithms (RBF-Support Vector Machines, Random Forrest, Logistic Regression) across 8 data sets of various complexity and instrumentation. All data sets were open access, downloaded from public online repositories (Metabolights & Metabolomics Workbench). All models were implemented in the Python 3 scripting language, presented using Jupyter Notebooks, and freely available on GitHub and mybinder.org. Results show that ANNs generally outperform the alternatives, particularly when the applied data sets are large. The ability to interpret ANNs have greatly improved since their first introduction thanks to advances in computer technology that allows the rapid use of methods such as network pruning and bootstrap resampling sensitivity analysis.

P-257 Retip: Increasing compound identification rate in metabolomics with a new Retention Time Prediction software in R

PRESENTING AUTHOR: ***Paolo Bonini, NGAlab, Spain***

CO-AUTHORS: ***Tobias Kind, Hiroshi Tsugawa, Dinesh Kumar Barupal and Oliver Fiehn***

Background: Untargeted metabolomics datasets in LC-MS/MS present a large number of unidentified peaks. Usually, less than 10% of all deconvoluted metabolic peak clusters are identified. Using retention times as orthogonal information is important in workflows for compound annotations because each orthogonal parameter increases the confidence level in compound identification. Methods: Five different machine learning algorithms have been employed and integrated into the Retip method: Random Forest, Bayesian Regularized Neural Network, Keras, XGBoost and LightGBM. Retention time prediction models have been developed for the two most common separation principles, reversed phase (RP) and hydrophilic interaction liquid chromatography (HILIC). We utilized two large publicly available dataset for training, the FiehnLab HILIC (n=981) and RIKEN PlaSMA (n=852) retention time databases. Results: For both chromatographic databases the XGBoost method was the most accurate with a root-mean-square error (RMSE) on the testing set of 1.14 min for HILIC and 0.69 min for RP. The Retip R package also significantly reduces the numbers of false positive compound’s by 26.5% and increases the correct identification rate by 21%. Retip has been integrated into the mass spectrometry software tools MS-DIAL and MS-FINDER allowing for a complete compound annotation workflow. Retip can be freely downloaded from the CRAN repository as an R package. Conclusion: Predicted retention times increase the confidence for peak annotations in liquid chromatography and subsequently lead to improved biological interpretations in metabolomics.

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POSTER SESSIONS 3 AND 4 – Wednesday and Thursday – all even number posters will be on display.

*AWARD WINNERS

NEW FRONTIERS

P-258 A single high-resolution mass spectrometry platform for targeted and untargeted lipid analysis revealed modification of lipids and discovery of novel lipids in Barley roots response to salt stress

PRESENTING AUTHOR: ***Thusitha Rupasinghe, Metabolomics Australia, Australia***

CO-AUTHORS: ***Dingyi Yu, Berin A. Boughton, Siria H.A. Natera, Camilla B. Hill, Pablo Tarazona, Ivo Feussner, Ute Roessner***

Untargeted and targeted lipidomics are two complementary approaches, which when combined can achieve in-depth and comprehensive lipidome characterisation and quantification. The two approaches are usually performed on two different Mass Spectrometer platforms, triple-quadrupole (QqQ) type MS and high-resolution MS. Here, we developed a robust lipidomics workflow merging both targeted and untargeted approaches on a single liquid chromatography coupled to quadrupole-time of flight system (LC-QqTOF) platform with scheduled parallel reaction monitoring (sPRM). sPRM assays integrate both untargeted profiling from MS1 scan and targeted quantification obtained from MS/MS data. With scheduled algorithm and maximum 100 precursors multiplexed per duty cycle, sPRM assay can achieve a high-throughput targeted monitoring of more than 250 lipid species within a single injection. This workflow enabled the identification and quantification of more than 700 lipid species from 25 lipid classes at the level of fatty acid/long chain base/sterol composition with three injections in a barley root extract. To further demonstrate the workflow, a study on salt stress induced changes in the barley root lipidome was initiated as well as the discovery of novel lipid species in the group of oligohexosylceramides. Results show that 232 targeted compounds and 888 unknown features were found to have significantly responded to salt stress. This emerging targeted and untargeted lipidomics strategy combined in a single workflow provides novel insights into lipidomics and its applications in addressing biological questions.

P-259 DIMEdb: A database/web service for metabolite identification in direct infusion mass spectrometery

PRESENTING AUTHOR: ***Keiron O'Shea, Institute of Biological, Environmental & Rural Sciences (IBERS), Aberystwyth University, United Kingdom***

CO-AUTHORS: ***Divya Kattupalli, Luis AJ Mur, Nigel W Hardy, Biswapriya B Misra, Chuan Lu***

Direct infusion mass spectrometery (DIMS) is a widely used platform that is capable of producing a a global fingerprint of the metabolome, without prior requirement of a chromatographic step - making it ideal for wide scale high-throughput metabolomics analyses. Metabolite identification is an arduous task that involves the querying of masses against a metabolite database in order to retrieve putative metabolite annotations. Each existing metabolite database differs in a number of aspects including coverage, format, and accessibility - often limiting the user to a rudimentary web interface with no means of programmatically interfacing with the database. Consequently, researchers often manually combine multiple search results for a single experiment in which the exploration of potentially hundreds of masses becomes an incredibly arduous task – and infeasible for many projects. To facilitate unified access to metabolite information we have created the Direct Infusion MEtabolite database (DIMEdb), a comprehensive web-based programmatic metabolite database that contains over 80,000 metabolites sourced from a number of renowned metabolite databases of which can be utilised in the analysis and annotation of DIMS data. DIMEdb is freely available at https://dimedb.ibers.aber.ac.uk.

P-260 Optimization of Caenorhabditis Elegans Homogenization and Extraction Methods for Non-targeted Metabolomics using Orbitrap and FT-ICR Mass Spectrometry

PRESENTING AUTHOR: ***Brianna Garcia, University of Georgia, United States***

CO-AUTHORS: ***Goncalo J. Gouveia, Bennett Fox, Franklin E. Leach III, Facundo M. Fernández, Frank Schroeder, I. Jonathan Amster, Arthur S. Edison***

Metabolomics commonly uses analytical techniques such as NMR and LC-MS to quantify and identify metabolites related to biological alterations. Metabolome coverage from non-targeted LC-MS studies relies heavily on the pre-analytical protocols used. These protocols impact which metabolites are successfully measured and thus, the biological conclusions drawn. Protocol standardization remains a challenge in the metabolomics community due to the variety of sample types analyzed and workflows used. Previous studies have demonstrated that homogenization and extraction methods can produce large variations in the total number of metabolites detected, precision and yield. To systematically determine the effects on metabolome coverage, artifact production, and experimental variation, different homogenization methods and extraction solvents (i.e. methanol, ethanol, 2:1 cholorform:methanol, and 9:1 ethylacetate:ethanol) have been examined using a mixed-stage C. elegans population. Sample preparation was completed by two individuals in triplicate and ran in each laboratory using an Orbitrap Q-Exactive HF to showcase both the intra-analyst and instrumental variance. This initial factorial design was used to narrow down the parameters that largely contribute to extraction efficiency. Using a Taguchi Design of Experiments method these parameters were further analyzed to determine the pre-analytical sample preparation that minimized artifact production, produced the highest metabolome coverage and the highest reproducibility. An L9-orthogonal array was used where extraction solvent, volume ratio, method of extraction, and reconstitution solvent represented the four factors. Future work includes MS/MS experiments utilizing Orbitrap and FT-ICR mass spectrometers to test the resolving power limits of the Orbitrap, establishing the minimum requirements for FT-ICR analysis of metabolomics samples.

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***AWARD WINNERS**

NEW FRONTIERS

P-261* A high-throughput method for obtaining microbial exometabolomics data using a 3D printed platform

PRESENTING AUTHOR: ***Caroline Birer, University of Pittsburgh, United States***
CO-AUTHORS: ***Rosalie K. Chu, Christopher R. Anderton, Erik S. Wright***

Microbial interactions are governed by an immense variety of small molecules. To date, our understanding of microbial communication is largely based on a small number of molecules used for quorum sensing. This limitation is partly explained by the low-throughput and high cost of most experimental approaches using mass spectrometry (MS). Here, we engineered a 3Dprinted device for eavesdropping on the exometabolome of pairs of microorganisms grown in interconnected environments. Our device can be used to easily co-culture microorganisms with or without additional stimuli (e.g. nutrients, drugs, etc.). Coupled with high-throughput robotics (e.g., Triversa Nanomate robot) and MS, the platform can be used to measure an exometabolome in 4minutes at a cost of only US$2. We have validated our approach with mixtures of 27known compounds at decreasing concentrations, where we were able to detect 79% and 17% of known compounds 10nmol and 10pmol, respectively. Furthermore, we applied the platform to study co-cultures of 5soil microbes at different time points after growth. Principle coordinates analysis (PCoA) revealed a high degree of repeatability, a transition of the exometabolome over time, and a clear signal species domination within the exometabolome. After subtracting mass spectrometry features related to media, we were able to pinpoint small molecules excreted by each individual microbe. This approach enables us to process an immense number of samples, unlocking previously insurmountable problems such as decrypting the higher-order principles of inter-cellular communication. Interpreting the language of the microbiome may bring us one step closer to manipulating microorganisms by communicating through small molecules.

P-262 Focused metabolomics based on TMT labeling for improving throughput and quantification

PRESENTING AUTHOR: ***Yoshiya Oda, The University of Tokyo, Japan***
CO-AUTHORS: ***Suzumi M Tokuoka, Yoshihiro Kita***

In metabolomics, the importance is placed on quantification rather than identification. Then, preforming quantitative analysis of many hundreds of samples is more important in metabolomics than identifying thousands of molecules in a single experiment, though proteomics often put emphasis on the latter. In quantitative analysis, stable isotope labeled substances are commonly used as internal standards for the target molecules to correct for variations during ionization. However, when there are many targets, it is not practical to prepare such internal standards for all targets. Improving multi-sample processing capacity and quantification in proteomics, isobaric tagging method so called iTRAQ or TMT method have been developed, in which MS/MS spectrum is used for relative quantification, iTRAQ/TMT is capable of relative quantification between the samples without isotope labeled internal standards by constantly adding one of the samples as a control. Despite this excellent method, it has hardly been used in metabolomics. One of the reasons is that the derivatization reaction is limited for the metabolite having a specific functional group such as a primary amine group. Metabolites having a carboxy group is preferred for derivatization, but in order to label the carboxy group, it has to be activated before labeling. Therefore, we investigated various derivatization conditions suitable for metabolomics. While finding some problems which have not been reported in proteomics, we came to apply to human plasma sample analysis. Our approach is not comprehensive metabolomics, but it is more practical method to perform quantitative analysis of hundreds of samples.

P-263 Single-platform metabolomic and proteomic profiling of human liver tissue

PRESENTING AUTHOR: ***Thierry Schmidlin, Dr. Margarete Fischer-Bosch - Institut für Klinische Pharmakologie, Germany***
CO-AUTHORS: ***Thierry Schmidlin, Kathrin Klein, Matthias Schwab, Thomas E. Mürdter, Ute Hofmann, Mathias Haag***

Metabolomic and proteomic profiling of liver tissue represents a powerful approach for the functional characterization of molecular processes underlying drug uptake and metabolism. However, the heterogeneous nature of tissue and the limited availability of biopsies demand for protocols that utilize samples in an economic fashion by simultaneously increasing information yield through recovering metabolites and proteins from a single sample. Here we demonstrate a strategy for the quantitative assessment of metabolites and proteins recovered from the very same liver tissue analyzed on the same MS platform. Samples were sequentially subjected to metabolite extraction and urea-based protein extraction protocols followed by tryptic digest. Metabolites were analyzed after HILIC separation in positive and negative ionization mode whereas peptides were monitored by C18-RP LC-MS/MS. Fragment spectra, acquired by data-dependent MS/MS measurements, enabled the structural assignment and identification of a broad range of metabolite classes and >1000 proteins, respectively. Over 75% of the annotated metabolic features exhibited CVs <25% in both ionization modes across replicates of independently prepared samples. Proteome quantification likewise showed high quantitative reproducibility, evidenced by a median intra-run correlation coefficient of 0.96 across all measurements. The approach allowed us to accurately monitor clinically relevant metabolites and related proteins such as bile acids and cytochrome P450 enzymes (CYPs) involved in their synthesis (e. g. CYP8B1 and CYP27A1). Applied to clinical cohorts the multi-OMICS approach will enable a deeper understanding of the interplay between proteins and metabolites associated with pharmacologically important phenotypes and hepatic diseases including non-alcoholic fatty liver disease.

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NEW FRONTIERS

P-264 Continous in vivo metabolism by NMR

PRESENTING AUTHOR: ***Michael Judge, University of Georgia, United States***

CO-AUTHORS: ***Yue Wu, Fariba Tayyari, Ayuna Hattori, John Glushka, Takahiro Ito, Jonathan Arnold, Arthur S. Edison***

Dense time-series metabolomics data are essential for unraveling the underlying dynamic properties of metabolism. Here we extend high-resolution-magic angle spinning (HR-MAS) to enable continuous in vivo monitoring of metabolism by NMR (CIVM-NMR) and provide analysis tools for these data. First, we reproduced a result in human chronic lymphoid leukemia cells by using isotope-edited CIVM-NMR to rapidly and unambiguously demonstrate unidirectional flux in branched-chain amino acid metabolism. We then collected untargeted CIVM-NMR datasets for Neurospora crassa, a classic multicellular model organism, and uncovered dynamics between central carbon metabolism, amino acid metabolism, energy storage molecules, and lipid and cell wall precursors. As an exploration of real-time perturbation of metabolism, we are also conducting aerobic-to-anaerobic shifts. Virtually no sample preparation is required to yield a dynamic metabolic fingerprint at ~1-min temporal resolution with little noise. CIVM-NMR is simple and readily adapted to different types of cells and microorganisms, offering an experimental complement to kinetic models of metabolism for diverse biological systems.

P-265 Discovery and identification of novel biomarkers for vitamin B6-dependent epilepsy through the combination of Next Generation Metabolic Screening and Infrared Ion Spectroscopy

PRESENTING AUTHOR: ***Karlien Coene, Radboud UMC Nijmegen, Translational Metabolic Laboratory, Netherlands***

CO-AUTHORS: ***Jonathan Martens, Giel Berden, Rianne E van Outersterp, Udo FH Engelke, Leo AJ Kluijtmans, Marleen CDG Huigen, Siebolt de Boer, Ed van der Heeft, Clara DM van Karnebeek, Ron A Wevers, Jos Oomens***

Antiquitin deficiency, also known as vitamin B6-dependent epilepsy, is an inborn error of metabolism affecting the degradation of lysine, which presents with severe epilepsy in newborns. The accumulating metabolite piperideine-6-carboxylate (P6C) complexes with active vitamin B6, causing a secondary deficiency of this co-factor for many enzymatic processes in the human body. Supplementation with large doses of vitamin B6 can overcome this problem in patients and resolve the epilepsy. However, treatment should start as early as possible to prevent irreversible damage to the neonate. Currently, no stable biomarkers in blood are available to include this disease in newborn screening. Using an untargeted metabolomics approach on a UHPLC-QTOF-MS set-up, which we have coined 'Next Generation Metabolic Screening', we have now identified novel biomarkers in plasma for antiquitin deficiency. However, for two of these markers, both with a neutral mass of 185.1052 but different retention times, chemical identity could not be established using the currently available metabolite databases. Through the application of infrared ion spectroscopy (IRIS)-MS, we were able to deduce the chemical structure of these unknown biomarkers, which were predicted to be two diastereomers of 6-(2-oxopropyl)piperidine-2-carboxylic acid. This identification could be confirmed by measurement of synthesized model compounds, which showed a perfect match to IR spectra and NGMS profiles of patient-samples. These novel markers are promising for application in neonatal screening to diagnose antiquitin-deficiency as early as possible. Our study has shown the power of untargeted metabolomics in combination with IRIS-MS to discover and identify novel biomarkers in inborn errors of metabolism.

P-266 CCSPredict: Using a machine learning approach for higher confidence in Lipid identification

PRESENTING AUTHOR: ***Ulrike Schweiger-Hufnagel, Chemist, Germany***

CO-AUTHORS: ***Sebastian Wehner, Heiko Neuweger, Sven Meyer, Aiko Barsch, Nikolas Kessler, Lucy A. Woods***

The use of ion mobility-featured mass spectrometers offers new options for higher confidence in annotations of target molecules. First, with the additional separation dimension compounds co-eluting from LC columns can be separated. The benefit is that a subsequent fragmentation will result in cleaner MS/MS spectra – crucial for any ID in lipidomics or other small molecule workflows. Moreover, ion mobility enables the determination of the collisional cross sections of ions. These values are specific properties for any ion species under given conditions (type of gas, pressure, temperature). Therefore, acquired values can be used for identification if they are compared to in-silico generated data or used in a library-based approach. We present a new tool for the prediction of lipid CCS values. It is fully integrated in MetaboScape 4.0 and is based on a machine learning approach1 that was extended significantly to cover a wider range of lipid structures. Predicted values can be compared with the ones measured on a Bruker timsTOF Pro instrument. The Trapped Ion Mobility Spectrometry technology enables the exact measurement of CCS values at a very high reproducibility and TIMS resolving power. Both are critical pre-requisites to make full use of the increased confidence by CCSPredict. This workflow helps to assign the structural classes of lipids.

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*AWARD WINNERS

NEW FRONTIERS

P-267 A novel integrative approach combining intelligent imaging acquisition and metabolomics reveals chemical signatures of human colon organoids.

PRESENTING AUTHOR: ***Josep Rubert, University of Trento. CIBIO - Dept. of Cellular, Computational and Integrative Biology, Italy***

CO-AUTHORS: ***Andrea Lunardi***

Colorectal cancer (CRC) is one of the most common cancers in the western world. On top of that, the forecasts show that the global burden of CRC is expected to increase by 60%, to over 2.2 million new cases and 1.1 million deaths by 2030, due to an aging population and western dietary patterns. Research has suggested that dietary patterns, dysbiosis, and microbial metabolites may play a pivotal role in CRC, leading to increasing interest among scientists. However, adequate in vitro models for deciphering the relationship between diet-microbiome-host are not yet available. The most common approach to study microbes and nutrients have involved the use of 2D cell lines. However, immortalized 2D cell lines differ metabolically from in vivo cells. Recently, human intestinal organoids have offered a better model system than 2D cell lines for cancer studies, providing 3D organization, multicellularity, and function. In spite of this, protocols are non-existent for organoids. We have recently outlined a novel integrative approach combining intelligent imaging acquisition and metabolomics. With this approach, colon organoids/tumoroids are seeded in µ-plate 96 well (Ibidi). This plate is suitable for growing 3D cell cultures, quantifying features such as the number, shape, texture, and size of organoids, and basic measurements, such as proliferation, DNA content (Hoechst 33342), viability and apoptosis. Subsequently, the media is aspirated and intracellular metabolites of human intestinal organoids/tumoroids are extracted and further analyzed by UHPLC-HRMS. In the future, we plan to investigate human colon organoid and tumoroid responses to gut microbial metabolites.

P-268 Characterisation of metabolically perturbed human stem cell derived dopaminergic neurons

PRESENTING AUTHOR: ***Alissa Schurink, Student at the LACDR, Netherlands***

CO-AUTHORS: ***Edinson Lucumi, Cornelius Willacey, Agnieszka Wegrzyn, Alida Kindt, Noëlle Bakker, Lalithasushma Chakravadhanula, Javier Jarazo, Jens Schwamborn, Amy Harms, Thomas Hankemeier, Ronan M.T. Fleming***

In Parkinson's Disease, it has been hypothesized that mitochondrial dysfunction leads to an imbalance in energy metabolism resulting in degeneration of substantia nigra dopaminergic neurons. We metabolically characterised the response of dopaminergic neurons, derived from human neuroepithelial stem cells, to chemical perturbations that targeted glycolysis and complex V of the electron transport chain. Exo- and endo-metabolomic samples were analysed with UPLC-MS/MS applying a novel targeted method based on the derivatization of amine, carboxy, and thiol groups. The inhibition of complex V resulted in a depletion of malate and succinate indicating a decrease in the TCA cycle. A significant increase in myristoylcarnitine levels indicated an increase in beta-oxidation, which may compensate for impaired ATP synthesis through complex V.

P-269 Bioengineering of Artificial Thymoma Spheroids as a 3D model for ex vivo/in vitro 3D drug investigation of toxicity employing novel microslot nuclear magnetic resonance technique

PRESENTING AUTHOR: ***Mohammad AlWahsh, Leibniz-Institut für Analytische Wissenschaften - ISAS - e.V., Germany***

CO-AUTHORS: ***Jörg Lambert, Ahmad Telfah, Mikheil Gogiashvili, Djeda Belharazem, Rosemarie Marchan, Alexander Marx, Roland Hergenröder***

The metabolic alteration of thymic epithelial tumors is poorly understood on both the tissue level and in vitro cell culture. The known potential of thymomas to undergo toxicity on exposure to chemotherapeutic agents may result in therapy failure. We are focusing on the pharmacokinetics and pharmacodynamics of anticancer drug therapy via metabolic profiling on living cells. Our special interest is the development of a 3D model of thymic carcinoma and life drug toxicity testing: The analysis of tumor self protection mechanisms will open new paradigms in cancer treatment. For metabolic profiling, we apply microstrip Nuclear Magnetic Resonance (NMR), suitable for ultra-small volume samples like 3D tumor models. We could successfully establish a dedicated microstrip detector for in vitro metabolic profiling of the spheroid cells as a function of their spatial position. We custom designed a Special microfluidic device, ensuring the viability of the cells. The metabolic variation of the well characterized thymic carcinoma cell line is determined using spheroids with and without drug anti-cancer drug treatment treatment. We could successfully establish 3D tumor models from thymic cell lines, using the "simple rotation technique". We here show the very first noninvasive toxicity testing employing NMR. We could visualize the penetration of the anti-cancer drug in a 3D tumor model. Even more, we were able to determine the concomitant response on a cellular level and we could deduce details on the drug transport efficiency and on drug target modications from these measurements.

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***AWARD WINNERS**

NEW FRONTIERS

P-270 Discovery of discriminative MR biomarkers from organoids for stratification of patients with gastrointestinal cancer

PRESENTING AUTHOR: ***Maria Tunset Grinde, Dept. of Circulation and Medical Imaging, Norwegian University of Science and Technology, Norway***

CO-AUTHORS: ***Maria T. Grinde, Wybe J.M. van der Kemp, Boudewijn M.T. Burgering, Siver A. Moestue, Madelaine van Macklenbergh, Hanneke van Laarhoven, Dennis Klomp, Hans Wienk, Jeanine Prompers, Tone Frost Bathen***

Introduction: Advances in in vitro 3D culture technologies, like organoids, have opened new opportunities for the development of physiologically relevant human cancer models. The aim of this study was to evaluate the quality of MR spectra acquired directly from organoids, thereby qualifying organoids as a tool for determination of discriminative metabolic biomarkers potentially transferable to the clinic. Materials and methods: Gastrointestinal cancer organoids (primary colon cancer, liver metastasis from esophageal cancer) were established as described [1]. Treatment of organoids with clinically relevant cytotoxic agents (Oxaliplatin, Fluorouracil, SN-38 or combinations of these), is ongoing. Organoids are analyzed with High Resolution Magic Angle Spinning (HR MAS) MRS, and by traditional HR MRS of the polar fraction after methanol/chloroform/water extraction. Results: We were able to identify approximately 40 metabolites from organoids and polar extracts. Metabolite concentrations in organoids and cell extracts were highly correlated (R2>0.91). 2D 31P/1H HSQC MR spectra from organoids and cell extracts display potential discriminative biomarkers like phosphocholine, glycerophosphocholine, phosphoethanolamine and glycerophoshpoethanolamine. Discussion: The results from this study show that we are able to acquire high quality MR spectra from organoids. MR spectra from organoids and polar cell extracts were highly correlated, indicating that metabolite extraction is not required to achieve high quality spectra. Use of 1D 1H and 2D 31P/1H HSQC MR spectra from organoids enables identification of potential discriminative biomarkers that can be used for stratification of patients with gastrointestinal cancer. References: 1. Sato, T., et al., Gastroenterology, 2011. 141(5): p. 1762-72.

P-271 Space-resolved sampling and metabolomics analysis of the oral cavity

PRESENTING AUTHOR: ***Alessio Ciurli, LUMC, Netherlands***

Saliva is a complex Biofluid that serves to maintain oral health through lubrification, clearance, buffering, antibacterial activity, taste, and digestion. Nowadays, high sensitivity of advanced analytical techniques, such as mass spectrometry, facilitate the analysis of saliva. Such analysis offers an enviable number of insights into health and disease of the oral cavity as well as into systematic alterations as for example hormone level. Saliva can be easily collected through non-invasive procedures such as direct split or use of swabs. Saliva is a mixture of different fluids secreted from three distinct salivary glands, namely, the parotids, the submandibulars, and the sublingual glands. Here we developed a novel sampling method with spatial sampling capability, allowing for the comprehensive metabolomics analysis of different oral biofilms originated from saliva secreted of different glands. In the present study, saliva samples were collected using micro-cellulose swabs in 5 different oral locations. Absorbed saliva was extracted and, subsequently, analyzed by our LC-MS/MS-based metabolomics platform (HILIC on Shimazdu Nexera LC-30 HPLC system – TripleTOF 6600 system ABSciex). Finally, the raw data were processed using MSDIAL and R Studio for further analyses and visualizations. PCA analysis displayed a peculiar metabolic profiling depending the oral location. The present outcomes display the potential of space-resolved metabolomic profiling in order to achieve a deeper understanding of the biochemical environment of the oral cavity and the distribution of oral disease, such as head & neck cancer or periodontal disease.

P-272 Use of metabolomics for exploring early mechanisms of hepatotoxicity triggered by amphetamine-like drugs

PRESENTING AUTHOR: ***Ana Margarida Araújo, UCIBIO, REQUIMTE, Faculty of Pharmacy of University of Porto (FFUP), Portugal***

CO-AUTHORS: ***Ana Margarida Araújo, Eduarda Fernandes, Maria de Lourdes Bastos, Félix Carvalho, Paula Guedes de Pinho, Márcia Carvalho***

Hepatic injury associated with amphetamine-like drugs has been extensively reported, but the mechanisms underlying liver damage caused at human relevant concentrations have not been fully elucidated. In this work, we used a sensitive non-target metabolomic approach based on gas chromatography-mass spectrometry (GC-MS) to identify the early adverse events caused by amphetamine-like drugs (MDMA, MDPV and methylone) in primary mouse hepatocytes (PMH). Intracellular metabolome analysis of PMH exposed for 24h to two subtoxic concentrations of each drug (corresponding to LC01 and LC10 levels) was performed. In order to establish the value of metabolomics to reveal drug toxicity mechanisms, traditional toxicological endpoints (i.e. MTT and LDH cell viability, GSH and ATP assays) were also evaluated. The results obtained by the multivariate analysis showed that each drug induces metabolome alterations in a concentration-dependent manner. Most of the altered metabolites belong to the class of amino acids, fatty acids, carbohydrates and carboxylic acids. The combination of metabolites and their respective levels was unique for each tested drug, being therefore useful in understanding their specific toxicological outcome and mechanisms of toxicity. Most importantly, our data provide compelling evidence that metabolomics is a suitable and even more sensitive approach when compared to conventional toxicological assays, as significant alterations in metabolite levels were already found at the lowest concentration tested. To the best of our knowledge, this is the first study highlighting the potential of the intracellular metabolome analysis to assess the early adverse events of drugs of abuse, providing new insights into their mechanisms of toxicity.

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*AWARD WINNERS

NEW FRONTIERS

P-273 NMR-based metabolomics: a novel tool to study stress in wild bird

PRESENTING AUTHOR: ***Karen Machin, University of Saskatchewan, Canada***

CO-AUTHORS: ***Asha Perera, Catherine Soos***

Metabolomics examines fluctuations in small metabolite levels in biological systems. An important application of metabolomics is the study of effects of natural and anthropogenic environmental stressors on organisms. The vertebrate stress response functions to re-route energy typically utilized for maintaining homeostasis, toward metabolic processes that provide energy available for immediate survival functions, thus affecting the metabolome at the cellular level. While multiple studies have successfully applied metabolomics techniques in invertebrate and mammalian species, the use of this technique in wild birds is still in its infancy. Our main objective was to validate the use of metabolomics in evaluating effects of stress on the metabolome of waterfowl. Captive lesser scaup (Aythya affinis) were implanted with either a biodegradable corticosterone pellet to mimic effects of chronic stress, or a placebo pellet. H1 Nuclear Magnetic Resonance (NMR) spectroscopy was performed on serum samples collected during the active implant period. We hypothesized that metabolomics can be used to differentiate ducks that received exogenous corticosterone from placebo (control). We found that serum metabolite profiles could be successfully used to differentiate ducks with higher serum corticosterone from control individuals. We further identified multiple key metabolites that varied between the two groups, all of which play important roles in energy metabolism. To our knowledge, this is the first study to investigate the use of NMR-based metabolomics techniques to study stress responses in a wild bird species. Metabolomic techniques may be a potential tool in identifying and characterizing metabolic responses associated with environmental stressors in wild birds.

P-274 Metabolic changes in murine hair follicles treated with procyanidin-B2 nutraceuticals by DI-MRMS

PRESENTING AUTHOR: ***Matthias Witt, Bruker Daltonik GmbH, Germany***

CO-AUTHORS: ***Eduardo Sommella, Emanuela Salviati, Christopher Thomson, Pietro Campiglia***

Known for anti-inflammatory and antioxidant properties, nutraceuticals enriched in Procyanidin-B2 promote hair growth both in vitro and in vivo. However, the metabolic changes associated with the treatment have not been elucidated. In this study, direct infusion magnetic resonance mass spectrometry (DI-MRMS) was employed to understand the metabolic shift produced by treatment with Procyaindin-B2 nutraceuticals (Annurca apple extract) in murine models. DI-MRMS allowed the identification of several metabolites using ultra-high mass accuracy and fast analysis time, glutaminolysis, pentose phosphate pathway, glutathione, citrulline and nucleotide synthesis derived metabolite were detected. The metabolic profile revealed that the treatment with Procyanidin-B2 results in the early exit of hair follicles from telogen phase and increased keratin biosynthesis.

P-276 Comprehensive Metabolomic and Lipidomic Profiling in Formalin-Fixed Paraffin-Embedded Human Kidney Tissue

PRESENTING AUTHOR: ***Sylvia Karin Neef, Margarete Fischer-Bosch-Institute of Clinical Pharmacology Stuttgart, Germany***

CO-AUTHORS: ***Heike Horn, Stefan Winter, Ute Hofmann, Thomas E. Muerdter, Elke Schaeffeler, German Ott, Matthias Schwab, Mathias Haag***

Nontargeted tissue metabolomics is a promising approach for the assessment of tumor-specific alterations and biomarker identification. In this regard, molecular profiling experiments could benefit from the opportunity to use clinical specimens archived as formalin-fixed, paraffin embedded (FFPE) tissue. We developed a method for nontargeted metabolomic and lipidomic profiling of FFPE tissue samples and carried out protocol optimization by systematic assessment of assay reproducibility (analytical precision and technical reproducibility) for structurally annotated metabolites. Comparison of porcine FFPE tissue with fresh-frozen samples revealed high overlap of metabolic features in organic (>90%) and in aqueous (>85%) extracts indicating high preservation of polar and nonpolar metabolites in FFPE tissue. Notably, a considerable proportion of features were exclusively detected in FFPE tissue which could in part be assigned as N-methylated and N-formylated phosphatidylethanolamine species indicating headgroup-specific modifications of lipids during fixation. Further assessment of tissue size and fixation time on analyte recovery revealed metabolite class-specific differences in their detection abundance. In this regard, the recovery of specific lipids (e.g. phosphatidylinositoles and acylcarnitines) remained largely unaffected thus rendering these molecules as potential biomarkers useful for future diagnostic applications. Finally, the applicability of the protocol was demonstrated by analyzing a set of clear cell renal cell carcinoma (ccRCC) and corresponding nontumorous FFPE tissue samples, achieving clear phenotypic distinction on the basis of their metabolomic profiles. Notably, metabolites found to be significantly altered between ccRCC and nontumorous FFPE samples showed considerable overlap (>20%) with metabolites previously identified to differ between ccRCC and nontumorous in fresh-frozen kidney tissue.

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NEW FRONTIERS

P-277 Semi-Targeted Metabolite Profiling for Improved Cellular Bioprocessing Outcomes Utilizing a Dual Separation/Mass Spectrometer with Intelligent MSn Acquisition

PRESENTING AUTHOR: ***Amanda Souza, Thermo Fisher Scientific, United States***

CO-AUTHORS: ***Ioanna Ntai, Anson Pierce, Paul Gulde, Martin Samonig, John Brann, Christopher Elicone, Ralf Tautenhahn, Daniel Lopez Ferrer, Andreas Huhmer***

Chinese hamster ovary (CHO) cells are often used for commercial production of recombinant therapeutic proteins. Optimization of cell culture medium and feeds is required to obtain maximum product yield. Metabolomics analysis allows for quick determination of nutrient limitations or metabolite buildup during cell culture. To better understand the metabolic events affecting cell biomass and antibody titer, we designed a semi-targeted workflow to confidently measure critical nutrients while allowing for the discovery of previously unidentified metabolites that affect growth and antibody yield. Separate cultures of CHO cells expressing the same recombinant antibody were supplemented with 6 different feeds and extracellular metabolites were sampled at different timepoints. Our analytical platform comprised of a dual UHPLC system for RP and HILIC separation of the same sample enabling increased analysis throughput while improving metabolome coverage, and the Thermo Scientific Orbitrap ID-X Tribrid MS, with AcquireX intelligent acquisition software, for maximizing the number of metabolites interrogated by MS/MS resulting in confident metabolite annotations. An in-house spectral library of 60 metabolites was constructed from authentic standards to ensure confident identification. Differential analysis of cell media provided robust metabolic indicators of cell growth and antibody yield. The semi-targeted approach rapidly detected amino acid and vitamin depletion for some of the feeds correlating to improved titer in shorter times. Overlapping two LC separations resulted in a 30% decrease in analysis time without compromising reproducibility or compound detection. This high-throughput semi-targeted workflow provided greater understanding for the rational design of cell media and feeds for improved commercial biopharmaceutical production.

P-278 An iterative approach integrating metabolomics with sensory science to improve the horticultural quality

PRESENTING AUTHOR: ***Victor Castro-Alves, Örebro University, Sweden***

CO-AUTHORS: ***Irina Kalbina, Åsa Öström, Åke Strid, Tuulia Hyötyläinen***

The integration between metabolomics with sensory studies can be a powerful tool to improve the overall taste and aroma of horticultural produce; however, improvements in sensory quality have been inferred mostly on changes of specific metabolites. It also is not entirely clear what is the relationship between what consumers regard as a crop with increased sensory quality and potential metabolites that influences its sensory attributes. Thus, we propose an iterative approach to increase the sensory quality of horticultural produce by applying comprehensive metabolic characterization by GC-Orbitrap and LC-QTOFMS and integrating the data both with sensory data and growing conditions using advanced bioinformatics tools. Each round of data correlation leads to suggestions of improvements in crop production, which were taken through cycles to arrive at an improved production protocol. To show the feasibility of this approach, we addressed the reduction of quality in herbs grown at greenhouses caused by the lack of UV exposure. Results show that when the herb dill is grown for 2 weeks with daily 4-hour supplementary UV-light, the horticultural produce improved 30% of its sensory quality from the situation at the outset towards the 'gold standard'. This data was linked with the phenolic compounds profile in the dill, thereby revealing the potential of integrating metabolomics with highly innovative testing for culinary aspects. Overall, this approach can lead to practical applications for the horticultural industry that benefits both producers and consumers, while also contributing to the basic understanding of the concept of crop quality.

P-279 Metabolomics analysis of high cell density erythroblast cultures: Small molecules trigger cellular oxidative stress

PRESENTING AUTHOR: ***Joan S. Gallego M., Delft University of Technology - Sanquin, Netherlands***

CO-AUTHORS: ***Joan S. Gallego M., Nurcan Yagci, Angelo D'Alessandro, Emile van den Akker, Aljoscha Wahl, Marieke von Lindern***

Transfusion-ready erythrocytes can be cultured from hematopoietic progenitors but at market-incompatible high costs. A limitation in maximum cell density, 2 x 10^6 cells/mL, has been observed during in vitro erythroblast expansion. Understanding the origin of this cell density limitation may provide strategies, both at media composition and feeding regime levels, to facilitate economically feasible upscaling. Growth-limiting conditioned media from proliferating cells cultured with varying levels of cell densities, 0.7 - 15 x 10^6 cells/mL, were generated. Media reconstitution experiments, performed by separating fresh media and growth-limiting supernatants using a 3kDa cut-off, and testing the effect of the filtrates and retentates on expanding erythroblasts, indicated that small molecules (<3kDa) are responsible for growth limitation. Targeted and untargeted metabolomics measurements during 36 hours of culture, for both the intra- and extracellular compartments, indicated progressive degradation of nucleosides and a strong depletion of essential lipids and amino acids in the medium, and an intracellular decrease in intermediates of the glutathione-ascorbate, γ-glutamyl and cysteine-methionine cycles. The latter pathways are involved in glutathione metabolism, a key intracellular antioxidant. Dilution of growth-limiting media suggests that, in addition to potential inhibitors, depletion of nutrients could be limiting growth. To identify potential depleted metabolites, growth-limiting media was supplemented with the components of fresh medium. Following a one-factor-at-a-time experimental design, components in the basal media IMDM were found to partially restore growth. Current experiments aim to optimize the medium and/or genetically adapt metabolic pathways to enable erythroblasts to grow at high cell density, and at low cost.

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NEW FRONTIERS

P-280 Ion mobility - MS based workflow for fast untargeted metabolite profiling

PRESENTING AUTHOR: ***Endre Laczko, FGCZ / ETH Zurich, Switzerland***

CO-AUTHORS: ***Junmin Hu, Sebastian Streb***

The potential of metabolomics to support personalised and translational, evidence based medicine is anticipated for quite some time. A variety of research laboratories have already demonstrated successfully that metabolomics can provide data to support personalized and evidence based medical decisions. However, no workflow was presented by the metabolomics research community yet which has the quality of point-of-care analytical testing approved and in use all over the world in medical practice. The lack of robust, reproducible and fast metabolite quantification workflows from start to end prevents metabolomics to step into day to day clinical applications. In this applied context, a metabolomics methodology has to fulfil several criteria: 1) Fast and robust metabolite extractions. 2) Fast and robust metabolite measurements and quantifications. 3) Fast and standardized data analysis. 4) Simple and intuitive data documentation. We have investigated in our laboratory the potential of ion mobility - mass spectrometry (IM-MS) workflows to close this quality gap. Here we like to present a point-of-care compatible IM-MS workflow as well as experimental data to demonstrate the speed and robustness of metabolite quantification by an untargeted metabolomics method.

P-281 A skin secretion metabolomic profiling of endemic Aegean species of Lyciasalamandra

PRESENTING AUTHOR: ***Sotirios Katsikis, University of Athens, Greece***

CO-AUTHORS: ***Karolos Eleftherakos, Eleftherios A. Petrakis, Leandros A. Skaltsounis, Maria Halabalaki***

Skin secretions of Amphibians are a unique and fascinating source of various bioactive natural compounds, like small peptides, alkaloids, and biogenic amines. In order to describe and explain differences between species, populations and specimens but also to find specific biomarkers, a metabolomics profiling of the skin secretion of the Greek endemic Lyciasalamandra helverseni and Lyciasalamandra luschani basoglui (Salamandridae family) was carried out. These species are found in the Aegean islands of Karpathos, Kasos and Kastellorizo, from where the samples were sourced. The metabolomics analysis was carried out using LC-HRMS (Orbitrap) and the pre-processing of data was performed with mzMine v.2.39 and XCMS v.1.5 with IPO parameter optimization. The preprocessed data were then used to create multivariate statistical models using SIMCA (v14.1). An evident separation in all taxon subdivisions was observed by employing unsupervised PCA. The supervised PLS-DA, OPLS-DA and O2PLS-DA models built were also able to reveal markers for identifying population differences. MS^n experiments of a selection of m/z were further utilized to assist with compound annotation, along with 2D NMR (HSQC and HMBC) of selected pooled samples. Evidence concerning novel elements of the species alkaloid arsenal is also presented. Acknowledgment: The present work was co-funded by the European Union (ERDF) and Greek national funds through the Operational Program “Competitiveness, Entrepreneurship and Innovation”, under the call “STRENGTHENING RESEARCH AND INNOVATION INFRASTRUCTURES” (project code: 5002803).

P-282 Assessing the use of Dried Blood Spot technologies for metabolomics research

PRESENTING AUTHOR: ***Nathan Lawler, Edith Cowan University, Australia***

CO-AUTHORS: ***Mary C Boyce, Stacey N Reinke, David I Broadhurst***

Typically, for blood-based metabolomics studies, whole blood is collected via venepuncture with plasma or serum being processed and biobanked for later analysis. This approach requires trained personnel and specialised laboratory equipment, so is generally limited to the laboratory or clinical setting. Moreover, venepuncture is often considered too invasive for applications requiring multiple repeat sampling within a short time. The use of dried blood spots (DBS) as an alternative collection method provides the advantage of reducing invasiveness, collection volume, and allowing collection to take place outside of the laboratory. Despite these advantages, there has been very limited adoption of DBS technology due to limited metabolite coverage, together with poor analytical stability and reproducibility. Recently, some of these limitations have potentially been resolved with improved collection technology; however, these methods have not yet been rigorously tested using high-resolution platforms. In this study, two different DBS technologies (hemaPen™ and hemaSpot™) were compared against traditional venepuncture. Blood samples were collected, using the three different methods, from six healthy adult participants. Samples were prepared and data were acquired using a standard blood LC-Orbitrap-MS workflow. Raw files were processed using Thermo Compound Discoverer v3.0. Metabolite coverage was assessed based on the number of identifications made using an in-house authentic chemical reference library as well as open-access spectral databases. Metabolite stability was assessed based on the percentage of metabolites present in DBS samples versus venepuncture samples. Analytical reproducibility was assessed using calculation of relative standard deviation of signal. The results of these comparisons will be presented.

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P-283 NMR in large-scale epidemiology

PRESENTING AUTHOR: ***Heli Salmela, Nightingale Health, Finland***

CO-AUTHORS: ***Salla Ruosaari, Peter Würtz***

Background: Advances in metabolomics now allow comprehensive biomarker profiling of entire biobanks and clinical trials. This provides a plethora of scientific opportunities such as discovery of novel biomarkers for onset of cardiovascular diseases and tracking their progression, as well as etiological insights into established cardiometabolic risk factors. Methods: Nightingale Health Ltd has developed a high-throughput metabolomics platform for population-wide initiatives and screening programs, and it is now being applied to profile close to 1.000.000 biobanked blood samples with extensive electronic health care records, including the entire collection of the UK Biobank with 500.000 samples. While reflecting the combined effects of lifestyle, environment and genetics, metabolomics provides a powerful tool for monitoring the health of individual patients or study participants over time. Here, we display the selection of the established and the most promising novel NMR biomarkers for disease events and risk prediction, focusing on how analyzing large cohorts facilitates biomarker discovery. We highlight cardiovascular diseases and diabetes, but also demonstrate how NMR metabolomics can be used in various smaller intervention studies and, for example, in research on gut microbiome and gut diseases. Conclusions: Here we showcase the benefits of metabolomic profiling in large-scale epidemiological settings for improved risk stratification. These results demonstrate how NMR metabolomics produces enhanced cardiovascular and diabetes risk prediction, allows novel means to track effectiveness of interventions and gives promise of future biomarker discovery for several chronic diseases.

P-284 Metabolites from human odour and textiles applying thermal desorption and GC×GC-TOFMS

PRESENTING AUTHOR: ***Paulina de la Mata, University of Alberta, Canada***

CO-AUTHORS: ***Sara Vaezafshar, Rachel H. McQueen, James J. Harynuk***

The relationship between human odour on different types of fabric is important in the textile industry because if differences in the generation and retention of body odour on fabrics can be controlled, it will be possible to make high performance garments that will require less frequent washing. Reduced laundering will extend the lifetime of the garment, and reduce the consumption of water and energy. The retention of molecules (metabolites) by fabrics depends on the distinct properties of the fabric, including its type and any applied finishes. Performance apparel is designed to draw moisture from the inside to the outside of the fabric for quick drying and wearer comfort. This study presents the results of an extensive investigation into the chemical analysis of metabolites of human body odour. T-shirt samples of five different fabric types worn by volunteers during at least one hour of physical activity for up to 20 wear and wash cycles were studied. Fabric samples were analyzed by direct thermal desportion (TD) of the fabric with separation and detection of the molecules effected by comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GC×GC TOFMS). Chemometric tools were used to study the differences observed between fabric types (cotton, polyester, and a cotton/polyester blend) and finishes on cotton fabrics.

P-285 Metabolic systems analysis of shock-induced endotheliopathy (SHINE) in Trauma – A new research paradigm

PRESENTING AUTHOR: ***Hanne Hee Henriksen, Section for Transfusion Medicine, Capital Region Blood Bank, Copenhagen University Hospital, Denmark***

CO-AUTHORS: ***Sarah McGarrity, Rósa S. Sigurðardóttir, Travis Nemkov, Angelo D'Alessandro, Bernhard O. Palsson, Jakob Stensballe, Charles E. Wade, Óttar Rolfsson, Pär I. Johansson***

Objective: Investigate the endothelial cell phenotype(s) that causes Shock-Induced Endotheliopathy (SHINE) in trauma. Background: We have studied more than 2,750 trauma patients and identified that patients with high circulating syndecan-1(endothelial glycocalyx damage marker) in plasma have an increased mortality rate compared to patients with lower levels. Notably, we found that patients suffering from the same trauma severity could develop significantly different degrees of endothelial dysfunction as measured by syndecan-1. Methods: Prospective observational study of 20 trauma patients admitted to a Level 1 Trauma Centre and 20 healthy controls. Admission plasma syndecan-1 level and mass spectrometry were measured and analyzed by computational network analysis of our genome-scale metabolic model (GEM) of the microvascular endothelial cell function. Results: Trauma patients had a significantly different endothelial metabolic profile compared to controls. Among the patients, four phenotypes were identified. Three phenotypes were independent of syndecan-1 levels. We developed GEMs representative of the observed phenotypes. Within these phenotypes, we observed differences in the cell fluxes from glucose and palmitate to produce Acetyl-CoA, and secretion of heparan sulfate proteoglycan (component of syndecan-1). Conclusions: We confirm that trauma patients have a significantly different metabolic profile compared to controls. A minimum of four SHINE phenotypes were identified, which were independent of syndecan-1level (except one phenotype) verifying that the endothelial response to trauma is heterogeneous and most likely driven by a genetic component. Moreover, we introduced a new research tool in trauma by using metabolic systems biology, laying the foundation for personalized medicine.

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NEW FRONTIERS

P-286 Advances and future perspectives in Eco-Metabolomics: From fingerprints to molecular mechanisms

PRESENTING AUTHOR: ***Kristian Peters, Biochemistry of Plant Interactions, IPB Halle, Germany***

CO-AUTHORS: ***Treutler H, Marr S, Balcke G, Döll S, Gorzolka K, van Dam NM, Neumann S***

Eco-Metabolomics is the application of metabolomics to ecology and biodiversity research to understand fundamental mechanisms regarding interactions of organisms with the environment, other organisms and within species communities. Ecological experiment designs that are often carried out in fields require a large number of samples to control the high variability of metabolite profiles. The focus on a broad range of non-model species that may never have been subjected to metabolite profiling before results in biochemically highly diverse profiles. Thus, major challenges include a wealth of data resulting from multi-level experiment design factors and a multitude of novel substances ("unknown unknowns"). Here, we give an overview on current challenges in Eco-Metabolomics and present major advances. The large data generated by Eco-Metabolomics experiments necessitate the integration of advanced statistical approaches to pinpoint metabolite features that explain the underlying research question most significantly. Feature selection methods can be used to perform subsequent identification only of the most relevant features using targeted in vitro methods like MS2/MS3, NMR or by applying in silico tools like MetFrag. Finally, we explain how annotating compound classes (e.g. using MetFamily and ClassyFire) represents a major breakthrough to describe molecular mechanisms in ecology and biodiversity research.

P-288 Ion Mobility Spectrometry paired with 13C Enrichment Strategies for Improved Metabolite Identification

PRESENTING AUTHOR: ***Robin Kemperman, University of Florida, United States***

CO-AUTHORS: ***Chris W.W. Beecher, Timothy J. Garrett, Richard A. Yost***

Ion mobility (IM) is a rapid gas-phase separation technique that can separate isobars that are unresolved by LC-MS. The combination of LC-IM-MS has shown great potential for metabolomics applications because of the ability to deconvolute complex samples and perform structure elucidation, in addition to calculate collision cross sections (CCS) of each metabolite based on their measured IM drift times (DT). In this study we have implemented a unique isotopic labeling technique into our LC-IM-MS metabolomics workflow for higher accuracy and confidence in metabolite identification. Isotopic ratio outlier analysis (IROA) uses the 13C enriched signal of all biochemically-synthesized metabolites in yeast, using a 5% or 95% 13C randomly labeled glucose in the cell media. IROA produces characteristic isotopologues which confirm biological origin, number of carbons, and removes artifacts. IROA is a powerful method for metabolic profiling; however, many isobars and isomers remain unresolved and the addition of numerous isotope signals increases the complexity of spectra. IM helps resolve overlapping IROA patterns and increases the confidence in metabolite identification; moreover, mass spectra show increased S/N ratios in the LC-IM-MS scans compared to LC-MS scans. Fragments of IROA-labeled metabolites maintain the IROA signature which aids in identification, however, due to low abundances are not always detected. For example, Arginine eluted at RT=0.7 min, with an appropriate IROA pattern (m/z range of 175.1196-181.1401); however, it's in-source fragments were hidden in the noise. After IM-filtering at DT=16.1 ms, 5 different IROA patterns belonging to arginine fragments were detected.

P-289 Derivatization of central metabolites in SUIT-2 cells using dimethylaminophenacyl bromide enabling LC-MS/MS energy-state analysis

PRESENTING AUTHOR: ***Cornelius Willacey, Analytical BioSciences and Metabolomics, Leiden University, Netherlands***

CO-AUTHORS: ***Martijn Naaktgeboren, Edinson Lucumi Moreno, Daan van der Es, Naama Karu, Ronan M. T. Fleming, Amy C. Harms, Thomas Hankemeier***

Recent advances in metabolomics have enabled larger proportions of the human metabolome to be analyzed quantitatively. A significant contribution to this field includes the chemical derivatization of metabolites and the use of isotope-coded derivatization (ICD). Chemical derivatization allows in principle a wide coverage in a single method, as it affects both the separation and detection of metabolites: it increases retention, stabilizes and improves the sensitivity of the analytes. Here, we describe a derivatization technique which simultaneously labels carboxylic acids, thiols and amines using the reagent dimethylaminophenacyl bromide (DmPABr). We further improve the quantitation by employing ICD, which uses internal standards derivatized with an isotopically-labelled reagent (DmPABr-D6). We demonstrate the ability to measure and quantify 64 central carbon and energy-related metabolites including amino acids, N-acetylated amino acids, metabolites from the TCA cycle and pyruvate metabolism, acylcarnitines and medium-/long-chain fatty acids. To demonstrate the applicability of the analytical approach, we analyzed SUIT-2 cells utilizing a 15-minute single UPLC-MS/MS method in positive ionization mode. SUIT-2 cells exposed to rotenone showed definitive changes in 28 out of the 64 metabolites, including metabolites from all 7 classes mentioned. This method provides broad coverage of the human metabolome and quantification of amines, carboxylic acids and thiol-containing metabolites.

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P-290 Educating the Next Generation – a Framework for Introducing HRMS and Metabolomics into Undergraduate Curricula

PRESENTING AUTHOR: ***Stacey Reinke, Edith Cowan University, Australia***

CO-AUTHORS: ***Nathan G Lawler, Yingqi Tu, Mary C Boyce***

Over the last decade the growth in metabolomics research has been exponential. This has been paralleled by increased educational opportunities at and above the postgraduate level. However, any acceleration in the knowledge economy inevitably creates a vacuum of essential skills in the next generation of potential scientists. Currently, topics such as high-resolution mass spectrometry (HRMS), cheminformatics and data science are extremely limited in most undergraduate education curricula. This needs to be rectified to ensure the stable growth of metabolomics. Implementing metabolomics content into curricula is challenging as it must both meet certain pedagogical requirements, whilst being integrated into a diverse number of existing undergraduate courses. For example, understanding HRMS needs to be embedded into chemistry curricula; pathway mapping into biochemistry curricula; spectral deconvolution and database searching into data science curricula. As a first step to addressing these issues, an authentic learning framework for the metabolomic analysis of blood, using HRMS, has been developed and effectively integrated into an undergraduate analytical chemistry course. The pedagogical aims were to (1) introduce students to the field of metabolomics and (2) enhance their understanding of HRMS, specifically the differences in processing and interpreting untargeted vs targeted data. A simple, easily replicated, experiment was devised to investigate the effects of hemolysis on the plasma metabolome, using horse blood to simulate human blood. Data were acquired using an LC-Orbitrap-MS, then processed and interpreted using XCMS-Online. This presentation will focus on the challenges and insights we gained in delivering complex, multi-disciplinary content at an undergraduate level.

P-291 Untargeted Metabolomics of Urine from Opium Users and Non-Users: A Golestan Cohort Study

PRESENTING AUTHOR: ***Susan Sumner, UNC Chapel Hill, Nutrition Research Institute, United States***

CO-AUTHORS: ***Reza Ghanbari, Wimal Pathmasiri, Susan McRitchie, Yuan Li, Arash Etemadi, Christian Abnet, Jonathan Pollock, Reza Malekzadeh***

The United Nations Office on Drugs and Crime estimated 34 million opioid users, and 19 million opiate users worldwide. Opiates are associated with health outcomes such as cancers, infectious diseases and psychological disorders. In the 1960s, research by Dole and Nyswander theorized that addiction is initiated through a metabolic disruption. Opium and derivatives can modulate gene expression, and impact neural and hormonal mechanisms, which can be reflected by metabolites excreted in urine. The Golestan Cohort Study, established in the northeast of Iran, includes more than 8,000 individuals who reported opium use with a mean duration of 12.7 years. For our study, over 500 urine biospecimens were selected for individuals who either reported no opium use (control), or reported opium use (maximum amount of opium eaten or inhaled per week), with/without tobacco. Untargeted analysis of urine was conducted using a UPLC-Q-Exactive-HFx-Orbitrap-MS. NMR data was acquired on a Bruker 700 MHz NMR. Data were modelled using logistic regression, multivariate analysis, and hypothesis testing with the phenotypes of non-user vs user, route and level of use, tobacco use, and opioid use disorder (OUD). Metabolite identifications and annotations were made using our RT, Mass, MS/MS in-house experimental physical standards library (Orbitrap data), Chenomx library (NMR), and Public Databases. We found perturbations in endogenous metabolites involved in one carbon metabolism, Krebs cycle, tryptophan metabolism, mediation of cell signal transduction, and neuroexcitatory activity. Metabolites of illicit drugs, tobacco products, carcinogens, phthalates, and plants also contributed to the phenotypes investigated. [Funding: 1U24DK097193, Sumner; NIDA INVEST Fellow, Ghanbari]

P-292 Coupling DAD and MS data in untargeted metabolomics

PRESENTING AUTHOR: ***Ron Wehrens, Wageningen University & Research, Netherlands***

CO-AUTHORS: ***Ron Wehrens, Jasper Engel, Rob van Treuren, Ric de Vos, Kenneth Haug and Philippe Rocca-Serra***

In untargeted metabolomics it is not uncommon to record UV/Vis absorbance data using a photodiode array detector (DAD) in addition to the mass spectrometry detector. DAD data can contain key information on the biochemical class or even structural identity of compounds exhibiting specific UV/Vis absorbance characteristics, like benzenes, plant pigments and Maillard reaction products. DAD data nowadays are typically used in a quality control context and are rarely seen as an autonomous source of information. As a result, no accepted standards for storing and exchanging data exist yet. Here we report on an open and general format for LC-DAD data based on the ISATAB standard, used in repositories like MetaboLights. This format allows the DAD data to be uploaded as a separate experiment assay. Motivating examples of multivariate DAD analyses and couplings with corresponding MS data are shown for a large LC-DAD-Orbitrap FTMS metabolomics data set on lettuce samples, available through MetaboLights. There are many possibilities for exploiting synergy between the two data layers. Since the chromatography of the two data layers is identical the DAD data can help in a better alignment of the more complex MS data, absorbance characteristics can be used to help annotating MS features, and in some cases quantitation of compounds may be easier in DAD data, too. When more and more DAD data sets become available, including injections of pure standards, multivariate analyses will become easier and more reliable, further enhancing the value of data dissemination through open platforms like MetaboLights.

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NEW FRONTIERS

P-293 A Room Temperature Metabolism Quenching Method for Automated Metabolomics Sample Preparation

PRESENTING AUTHOR: *Genevieve Van de Bittner, Agilent Technologies, United States*

CO-AUTHORS: *Alex Apffel, Kristin B. Bernick, Manuel Gomez, Brian P. Smart, Christine Miller, Steven Fischer, Laurakay Bruhn*

Many existing methods for cell-based metabolomic sample preparation require low-temperature metabolism quenching procedures to prevent interconversion of metabolites in a rapid, controlled manner. The low temperatures required to quench metabolism often utilize cold liquids, such as liquid nitrogen, which are difficult to handle and store. Additionally, low temperatures complicate automation of metabolism quenching steps, since they promote collection of condensation on robotic components, which can hinder robotic movement and cause rusting. We developed a mass-spectrometry-compatible method for room temperature metabolism quenching and simultaneous cell lysis to avoid the issues associated with low temperature quenching. A metabolic flux analysis utilizing this room temperature quenching method confirmed rapid metabolism quenching with no detectable conversion of 13C-labeled glutamine, added in the quenching and lysis buffer, to glutamate or other TCA cycle intermediates. Adenosine triphosphate stability testing indicated metabolite stability was maintained for at least a day at room temperature. We have integrated the room temperature quenching method into a complete sample preparation workflow that includes protein and lipid removal steps, which have been optimized for metabolite recovery, to provide a clean metabolite sample for LC/MS analysis. Targeted and untargeted LC/QTOF analyses of mammalian-cell-derived samples indicate our new sample preparation workflow extracts key central carbon metabolites and provides a wide coverage of the metabolome. Our room temperature quenching method simplifies the time-critical portion of the metabolomics sample preparation process, enables robust automation, and fills an important need in the metabolomics community.

P-294 Novel MALDI imaging solution empowered by a MALDI-Q-TOF and dedicated bioinformatics pipeline for identification of metabolites and lipids from tissue

PRESENTING AUTHOR: *Alice Ly, Bruker Daltonik GmbH, Germany*

CO-AUTHORS: *Janina Oetjen, Michael Becker, Richard R. Drake, Anand Mehta, Lucy Woods, Shannon Cornett, Alice Ly*

MALDI Mass Spectrometry Imaging (MALDI-MSI) has emerged as a technique to spatially resolve different metabolic processes in tissue sections. We present a novel workflow solution consisting of a high spatial resolution MALDI source and stage mounted on a commercially available QTOF. A range of metabolomics imaging applications will be highlighted. This includes measurements of endogenous metabolites; typically these measurements were previously performed using extremely high mass resolving instruments. Measurements of mouse intestine sections revealed differential abundance of multiple endogenous metabolites localized to different anatomical regions. Kidneys from rats treated with substance Factor Xa antagonist and untreated rats were analyzed for the distribution of the compound, compound metabolites and lipid signals. The compound (m/z 432.15) and associated metabolites were detected in the renal medulla; neither were detected in the non-treated samples. A number of lipid signals were increased or decreased in both the renal cortex and medulla of compound-treated animals when compared to those not receiving treatment. When combined with a software processing pipeline for automatically annotating measured ions, this enabled generation of annotated images from MALDI-imaging data. Glycoconjugates can be measured as metabolic endpoints of glucose metabolism. N-glycan imaging was conducted on human liver carcinoma sections and results compared against a list of glycans generated using a high-mass-resolving MRMS system. 61 out of 61 N-linked glycans signals were detected in the sample measured on the MALDI-QTOF, with clear differences in tumor versus non-tumor regions. These results demonstrate the capability of our MALDI-QTOF instrument for the MALDI-MSI of different metabolomics applications.

P-295 New discovery from CE-FTMS based metabolome profiling applied into fasting rat

PRESENTING AUTHOR: *MOON-IL KANG, Human Metabolome Technologies, Japan*

CO-AUTHORS: *Kazunori Sasaki, Kaori Abe, Makoto Suzuki, Satoshi Ito, Tsutomu Negama, Kenjiro Kami*

New unique platform, CE-FTMS, Capillary Electrophoresis coupled with a Fourier Transform Mass Spectrometer, been established recently by our patent electrospray ionization (ESI) source adaptation. In the application study with metabolomic profiling of multiple organ samples of fasted rats, we aimed to evaluate analytical performance whether CE-FTMS can be used as improved method compare to CE-MS for the purpose of analyzing charged metabolites comprehensively. Here we report an overall appearance for plasma and soleus muscles in collected all 5 different organs with 5 time-course points for 24 hours (n=5, each for 0, 4, 8, 16, and 24-hr) from fasted rats. CE-FTMS platform resulted in successful detection of 323 and 395 metabolites in plasma and soleus muscle separately, showed the increased detection number more than 1.7-fold or 1.8-fold compared to CE-MS. Especially, 2-fold increase of detection number in anion mode of CE-FTMS at both plasma and soleus samples. In spite of the significant difference in detection numbers between CE-FTMS and CE-MS, the movement of samples from starvation start (0 hr) to 24 hrs by CE-FTMS is pretty much the same as the CE-MS in PCA plot analysis, considering contribution of PC1 and PC2. Moreover, one third of metabolites within top or bottom 30 list of factor loading of PC1 and PC2 in CE-FTMS has not identified in CE-MS. We also confirmed that several metabolites showing partly in CE-MS at the start or end of fasting time such as cytosine, S-methylmethionine, spermidine, and thymidine are identified every samples in CE-FTMS measurement.

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*AWARD WINNERS

NEW FRONTIERS

P-296 The NIH Metabolomics Consortium

PRESENTING AUTHOR: ***Mike Conlon, University of Florida, United States***

CO-AUTHORS: ***Padma Maruvada, Richard A. Yost***

The NIH Common Fund Metabolomics Program has established a new consortium with the overall goal to realize the potential of metabolomics to inform basic, translational, and clinical research by (1) establishing an enduring national public repository for metabolomic data, (2) overcoming technical hurdles in analyzing and interpreting metabolomics data, including the ability to determine metabolite identities, and (3) developing consensus for, and promoting adoption of, best practices and guidelines to promote accuracy, reproducibility, and re-analysis of metabolomics data in collaboration with the national and international communities. This presentation will provide a perspective on the consortium, and explore the opportunities to engage stakeholders and the international metabolomics community.

P-297 In silico metabolite property libraries and quantitative chemical space analysis: a path toward novel molecule identification and false discovery assessment

PRESENTING AUTHOR: ***Ryan Renslow, Pacific Northwest National Laboratory, United States***

CO-AUTHORS: ***Sean Colby, Jamie Nuñez, Yasemin Yesiltepe, Niranjan Govind, Dennis Thomas, John Cort, Justin Teeguarden, Karen Wahl, David Wunschel, Thomas O. Metz***

Characterization of chemical “dark matter” is critical for human metabolome and exposure assessment, but the identity of many biologically-active compounds will remain unknown until analytical chemistry approaches are developed that (i) can identify compounds without reliance on data from analysis of authentic reference materials, (ii) have resolving power sufficient to resolve chemically similar compounds and (iii) have methods for comparing experimental data to reference libraries of chemical properties while accounting for the inherent accuracies in aspects of all associated analytical and computational pipelines. To begin addressing this need, our team has developed a suite of tools that collectively assembles evidence for the presence of small molecules in complex samples through the use of libraries containing multiple measured and calculated chemical properties. Here, we discuss the advancement of these tools and a current evaluation of this suite of standards-free identification methods on a set of natural products and microbial toxins. Furthermore, we evaluated four existing and new methods for quantifying chemical space: (i) principal components calculated from over 50 chemical properties, (ii) K-means clustering on these properties, (iii) distribution of ClassyFire superclasses, and (iv) relative location in deep learning latent space. The results show that compounds with similar behavior (e.g. detectable via MS) are best grouped using clustering, rather than by overall chemical class. Finally, we discuss how these methods may enable determination of a conservative false discovery rate for molecular identification and lead to generation of novel candidate molecules that do not correspond to any currently known molecules.

P-298 High speed untargeted 4D-lipidomics and -metabolomics LC-MS/MS workflows with Parallel Accumulation Serial Fragmentation (PASEF)

PRESENTING AUTHOR: ***Sven Meyer, Bruker Daltonik GmbH, Germany***

CO-AUTHORS: ***Ulrike Schweiger-Hufnagel, Aiko Barsch***

A crucial step in lipidomics and metabolomics studies is the acquisition of MS/MS data. To reliably identify analytes, the MS/MS fragmentation has to be fast enough to generate a reasonable number of high quality spectra over a chromatographic peak. This becomes even more complicated if the LC run times are very short. While there is an in-depth oriented approach to ID as many lipids as possible, clinically-oriented projects often demand a high-throughput for large sample cohorts. Therefore, a short cycle time per sample is needed to realize studies with hundreds or even more samples in a reasonable time frame. In order to keep up with this, the analytical instrumentation needs to deliver a high data quality at high acquisition speeds. This is realized by the PASEF (Parallel Accumulation Serial Fragmentation) acquisition mode. For this presentation, extracts of the NIST reference standard SRM 1950 were investigated for the number of identified lipids using different LC run times. The benefit of the additional ion mobility separation is to get clean MS/MS spectra of co-eluting compounds, e.g. isobaric lipids. This is demonstrated on PC / PE lipids. Furthermore, the ability to reliably identify differences between sample groups, even at short LC run times will be presented.

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PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-299 Cytoprotective Effects of Wheat Sprout Extracts on tert-Butyl Hydroperoxide-Induced Oxidative Stress in HepG2 Cells

PRESENTING AUTHOR: ***Hyeonmi Ham, National Institute of Crop Science, Rural Development Administration, South Korea***
CO-AUTHORS: ***Hye-Young Seo, Hyun Young Kim, Woo Duck Seo, Mi Ja Lee, Ki-Chang Jang***

Wheat sprout contains various ingredients that are beneficial to health. It is reported that wheat sprout has antioxidant, hypoglycemic, anticarcinogenic, and anti-inflammatory effects. Because of these health-promoting properties, wheat sprout is widely used in juice or tablet form. However, the intracellular antioxidant mechanisms of wheat sprout are still poorly understood. The objective of this study was to evaluate the protective effects of wheat sprout extracts against tert-butyl hydroperoxide (TBHP)-induced oxidative stress in HepG2 cells. The levels of cellular reactive oxygen species (ROS), glutathione (GSH), malondialdehyde (MDA), and antioxidant enzyme activities were investigated as biomarkers of cellular oxidative status. HepG2 cells were treated with various concentrations of wheat sprout extracts (0~50 μg/ml) prior to treatment with TBHP for 4 h. The incubation of HepG2 cells with TBHP led to decrease in cell viability. However, pretreatment of the cells with the wheat sprout extracts rescued cell viability in a dose-dependent manner. Generation of reactive oxygen species (ROS) induced by TBHP treatment in HepG2 cells was markedly ameliorated after treatment with wheat sprout extracts. Cellular depletion of GSH and formation of MDA were reduced by wheat sprout extracts. Moreover, pretreatment of wheat sprout extracts prevented a significant increase in catalase and glutathione reductase induced by TBHP. These results showed that treatments with wheat sprout extracts protect the cells against oxidative stress by modulating ROS production, GSH level, MDA generation, and antioxidant enzyme activities in HepG2 cells.

P-300 NMR-based Metabolomics: A More Sensitive Bioindicator of Stream Ecosystem Health

PRESENTING AUTHOR: ***Robert Brua, Environment and Climate Change Canada, Canada***
CO-AUTHORS: ***Adam G. Yates, Joseph M. Culp***

Human modification of landscapes and subsequent degradation of aquatic ecosystems is a global threat to the ecological integrity of waterways. This pervasive threat is evident in southern Manitoba, Canada where intensive agriculture has led to nutrient enrichment and habitat degradation of waterways. In highly modified landscapes, there is great difficulty in assessing the ecological health of aquatic systems as few unimpaired sites exist and stream communities, such as benthic macorinvertebrates, traditionally used in biomonitoring have become more homogeneous. Indeed, traditional bioassessment approaches have been unable to link human activities, such as the extent of agriculture in the watershed, and ecological condition of streams in southern Manitoba. Therefore, a critical need exists to identify alternative techniques for assessing stream ecosystem health. We used NMR-based metabolomics to evaluate the suitability of the metabolome of northern crayfish (Faxonius virilis) as a bioindicator of environmental exposure to stressors associated with agricultural and rural land use activities. In contrast to past assessments using benthic macroinvertebrate composition, the metabolome of crayfish tail tissue was able to differentiate among streams with high, medium and low amounts of agriculture in the watershed. Moreover, metabolite changes indicated stressor exposure altered energy metabolism pathways. These results suggest the metabolome of crayfish tail tissue is a more sensitive indicator of human impacts on stream ecosystem condition in highly altered landscapes. We conclude the use of NMR-based metabolomics to assess aquatic ecosystem condition should be encouraged. We also recommend additional research to develop protocols and assessment models for aquatic biomonitoring using metabolomics.

P-301 Prenatal chemical exposure modifies neonatal metabolome and type 1 diabetes risk

PRESENTING AUTHOR: ***Tuulia Hyötyläinen, Örebro University, Sweden***
CO-AUTHORS: ***Aidan McGlinchey, Tim Sinioja, Santosh Lamichhane, Johanna Bodin, Heli Siljander, Dawei Geng, Cecilia Carlsson, Daniel Duberg, Jorma Ilonen, Suvi M. Virtanen, Hubert Dirven, Hanne Friis Berntsen, Karin Zimmer, Unni C. Nygaard, Matej Orešič, Mikael Knip***

In the last decade, the increasing incidence of type 1 diabetes (T1D) stabilized in Finland, coinciding with tighter regulation of per- and polyfluoroalkyl substances (PFAS). In a mother-infant cohort study (n=264), we analyzed metabolite profiles of pregnant mothers and their offspring at birth with UHPLC-QTOFMS and GC-QTOFMS (260 identified lipids and metabolites) and quantified 22 PFAS in maternal samples during pregnancy (UHPLC-QqQMS). We then further examined the impact of PFAS exposure on metabolome in non-obese diabetic mouse (NOD) model. High PFAS exposure during pregnancy was associated with altered metabolic profiles, particularly with decreased phospholipids in the offspring. This association was exacerbated with increased human leukocyte antigen-conferred risk of T1D in infants. Importantly, we observed a remarkable similarity between the metabolic signature observed in the current study and the known signature associated with progression to T1D (Oresic et al, 2013). The NOD model verified our findings showing that prenatal exposure to PFAS resulted in a similar decrease in phospholipids also in the mice offsprings. We conclude that high PFAS exposure may alter sphingolipid levels during fetal development which may then go on to play a pathogenic role in the development of T1D later in life. Our data also highlight a potential role for a gene-environment interaction, which may lead to altered lipid profiles in newborn infants at-risk of developing T1D. Taken together, our findings suggest that high PFAS exposure during pregnancy contributes to risk and pathogenesis of T1D in children.

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PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-302 Xenometabolome of Mussel Exposed to Environmental Contaminants Mixtures in Field Conditions

PRESENTING AUTHOR: ***Diana Álvarez-Muñoz, Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Spain***
CO-AUTHORS: ***Álvarez-Muñoz Diana, Lidia Molina, Marinella Farré***

In our daily life activities, thousands of chemicals compounds are released to aquatic the environment. They can make their way first to river water and later on to sea water. An interaction between the contaminants present in the aquatic media and the resident organisms may occur and some of them can be bioaccumulated. Besides the importance of analyzing changes in the metabolome for understanding the effects of contaminants mixtures, it is also important to identify the xenobiotics accumulated that might be responsible of these alterations. In order to study this a metabolomics approach was undertaken in mussels collected from Ebro Delta (Spain). The approach followed has allowed profiling the xenometabolome or exposome. A field experiment was carried out and mussels from a shellfish farm allocated outside the Delta were transplanted into several sampling points for 1 month. After this period, they were collected, haemolymph was extracted, centrifuged, snap frozen and kept at -80 °C until its analysis. Prior injection in Orbitrap-Q-Exactive it was diluted 1:1 with methanol and filtered through a phospholipid removal plate. A non-target approach was followed by using Compound Discoverer 2.1. The differential analysis was carried out by using the organisms from the "clean site" as a control group, and the organisms from the other sampling points as exposed groups. Data sets were also exported to the statistical software "R" for multivariate analysis. Preliminary results show bioaccumulation of pesticides such as bentazone, pharmaceutical compounds like venlafaxine, and other compounds with endocrine disrupting properties (i.e. triclosan and methylparaben).

P-303 Metabolic foot-printing approach to study long term environmental impacts of biological and synthetic insecticides

PRESENTING AUTHOR: ***Cédric BERTRAND, AKINAO / CRIOBE / UPVD /, France***
CO-AUTHORS: ***Chandrashekhar Patil, Amani Ben Jrad, Hikmat Ghosson, Delphine Raviglione, Marie-Virginie***

Mosquitoes can carry infectious diseases from person to person and from place to place. Presence and establishment of invasive mosquito species such as Aedes aegypti and Aedes albopictus is rapidly increasing in the European environment. The European Directive in 1998 led to the increasing use of biological insecticides such as cry proteins produced by the bacterium Bacillus thuringiensis israelensis (Bti) that kill mosquito larvae after being ingested. Considering the interest in Bti as more environmentally sustainable bioinsecticide, it is important to examine in detail environmental fate and impact of Bti. The available tool such as half-life, t1/2, does not considers the biodegradation and biotransformation phenomenon of complex formulations. To address this challenge, 'Environmental Metabolic Footprinting' (EMF), giving an idea of the resilience time was recently developed in the laboratory (Patil et al. 2016; Salvia et al, 2017) to evaluate the impact of synthetic, botanical and microbial insecticides on soil and sediment matrix. The project 'EnvFate' aims to employ an EMF approach, to dynamically characterize environmental markers of Bti pollution found among the sediment matrix meta-metabolome. Metabolome characterization will require to develop and optimize extraction and detection protocols using LC-MS platform. In addition, metabarcoding approach will allow to understand microbial community responses to the Bti pollution. We performed a year long experiment to know the changes in the meta-metabolome after Bti and α-cypermethrin treatment in the three different sediment matrix. These activities pave the way for the development and adaptation of new environmental monitoring tools.

P-304 Integration of non-target screening and effect-based monitoring to assess OMP related water quality changes in drinking water treatment

PRESENTING AUTHOR: ***Andrea Mizzi Brunner, KWR Watercycle Research Institute, Netherlands***
CO-AUTHORS: ***Cheryl Bertelkamp, Milou Dingemans, Annemieke Kolkman, Bas Wols, Bram Martijn, Ton Knol, Thomas ter Laak***

The ever increasing production and use of chemicals augment their occurrence in drinking water and its sources so that monitoring using targeted chemical analyses alone is no longer sufficient. High-resolution mass spectrometry (HRMS) based non-target screening (NTS) has therefore become a promising tool to assess chemical water quality as well as the changes thereof during water treatment. However, to date the high number of features, i.e. unique combinations of accurate mass and retention time, resulting from NTS renders structural identification virtually impossible and prioritization is required. Here, we developed an approach to interpret and prioritize features based on the integration of chemical and effect-based data with data science methods, and applied it to the assessment of water quality changes in drinking water treatment trains at three pilot installations. These installations included advanced oxidation processes, ultrafiltration with reverse osmosis, and granular active carbon filtration. A selection of organic micro-pollutants relevant for the drinking water sector was spiked into the water treated in these installations. LC-HRMS based NTS and bioassays that enable the monitoring of biological effects of complex mixtures of chemicals were performed. NTS data was screened for predicted and known transformation products of the spike-in compounds. Patterns and trends were evaluated using multivariate analysis methods. This allowed for efficient visualization of the complex data. By integrating the chemical NTS data with the biological effect-based results potential toxicity was accounted for during prioritization. Together, the developed workflows allowed to monitor water quality and changes in water quality during water treatment.

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PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-305 Integrative omics analysis reveals resource partitioning strategies during toxin production in Microcystis aeruginosa

PRESENTING AUTHOR: ***Shruti Pavagadhi, National University of Singapore, Singapore***

CO-AUTHORS: ***Di Pan, Shivshankar Umashankar, Amit Rai, Peter I. Benke, Megha Rai, Gourvendu Saxena, Vamshidhar Gangu, Sanjay Swarup***

Rapid urbanization and climate change have contributed to an increased incidence of harmful algal blooms (HABs) in natural waters. HABs caused by Microcystis aeruginosa produce toxic secondary metabolites, such as microcystins (MCs). To understand resource partitioning strategies between cell growth and MCs production in response to environmental triggers, we tested the effect of interactions between light and nitrogen on Microcystis aeruginosa PCC 7806 using integrative omics. We measured the molecular changes to light and nitrogen combinations at the transcriptome and metabolome levels. We mapped these to networks of cellular processes thereby identifying overall strategy for resource re-allocation. Statistical models revealed that both light and nitrogen affected the transcriptome and metabolome, however, light was the major factor driving the changes at transcriptome level, when compared to nitrogen. On the contrary, effects at metabolome level were less pronounced and were restricted to a few inter-related networks that share rate-limiting substrates and pathway intermediates. Through the integrative systems level approach, we found certain networks belonging to cellular growth, cellular scavenging and secondary metabolite metabolism that drive the overall resource partitioning strategy in Microcystis. Additionally, two rate limiting metabolites (malonyl-CoA and metabolite couplet SAM; S-adenosyl methionine–SAH; S-S-Adenosyl-L-homocysteine) that occur at the crossroads of the light and nitrogen dependent pathways were identified, thus controlling the metabolic flux and resource allocation in Microcystis cultures. Through these observations, we provide a conceptual model for environmental responses of Microcystis. This model could be validated through controlled feeding experiments and specific genetic mutants to obtain further insights.

P-306 Combining acute toxicity, toxicokinetics and metabolomics approaches for comprehensive toxicity assessment of xenobiotics in aquatic organisms - Zebrafish embryos exposed to triclosan as a case study

PRESENTING AUTHOR: ***Dimitrios Damalas, Laboratory of Analytical Chemistry, University of Athens, Greece***

CO-AUTHORS: ***E. Panagopoulou, M. Agalou, D. Beis, M.H. Lamoree, P.E.G. Leonards, N.S. Thomaidis***

The ever increasing contamination of the aquatic environment from xenobiotics has raised concerns, regarding their adverse effects on aquatic organisms. To evaluate the impact of toxicants in depth, the whole xenometabolome and endometabolome of aquatic organisms should be studied. Zebrafish provides a promising alternative model for toxicological studies. The overall goal of this study was to highlight a high-throughput testing strategy, incorporating data from different approaches (fish embryo toxicity, toxicokinetics and metabolomics) for a comprehensive toxicity assessment of xenobiotics in aquatic organisms. Zebrafish embryos exposed to triclosan (TCS) were used as a case study. Initially, potential acute toxicity of TCS in zebrafish embryos was assessed. Afterwards, the uptake and biotransformation of TCS by zebrafish ebryos were evaluated. The final objective was to establish a wide-scope targeted metabolomics screening workflow to investigate the induced toxicity in a biochemical perspective and associate the observed toxicity/phenotype with changes at molecular level. To cover the wide polarity range of xeno- and endometabolome, a platform with broad analytical coverage was developed, combining RPLC and HILIC with ESI-HRMS. Suspect and non-target screening workflows were applied for the identification of biotransformation products. Regarding the metabolomics part of the study, a database of 600 endogenous metabolites was established, covering a broad range of primary metabolism. This approach is an alternative strategy to the classic targeted methods, as it did not focus on a few metabolic pathways, for which we already know that are affected by the specific stimulant. The proposed strategy unravels the involvement of unexpected metabolic pathways.

P-307 An Omics Approach Towards Identification of Unknown Emerging Pollutants in Marine Waters

PRESENTING AUTHOR: ***Steve Huysman, Ghent University, Belgium***

CO-AUTHORS: ***Francis Vanryckeghem, Kristof Demeestere, Lynn Vanhaecke***

The potential prevalence of emerging organic micropollutants in the aquatic environment has become a significant risk for public and ecological health, which is stressed by different regulatory bodies. Despite this, legislation so far mainly focuses on a few priority pollutants, thereby missing a substantial fraction of the known and unknown compounds occurring in water. Hence, the prevalence and identity of most compounds remains unexplored. Therefore, this study presents a novel analytical strategy for elucidating structurally related emerging organic pollutants in the aquatic environment. In-house developed and validated UHPLC-HR-Q-Orbitrap-MS methods targeting 70 steroids (Huysman et al., 2017) and 27 plasticizers (Huysman et al., 2019) were used to enable the detection of 1292 unique unknown emerging organic pollutants in 24 seawater samples (obtained from the Belgian part of the North Sea). To elucidate typical fragmentation profiles and identify characteristic fragments of the above-mentioned chemical pollutants, the target standards were respectively fragmented at 35 and 20 eV. As a result, typical fragmentation profiles were obtained for the steroids and plasticizers. The generated fragments of the unknowns were screened – using a newly written Python code – on their agreement with characteristic fragments and neutral losses obtained from the target standards. In total, 13% (n=173) of the unknowns present in water could be tentatively identified, i.e. 125 steroids, 21 alkylphenols and 27 phthalates, at a confidence level of Tier 3. Finally, during untargeted screening of seawater samples, all target analytes were successfully detected, confirming that fragmentation occurred also in a salty matrix.

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PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-309 Lipid changes in different tissues of rats after chronic exposure to ambient fine particulate matter

PRESENTING AUTHOR: *Ting-Zhen Chen, Institute of Environmental Health, National Taiwan University, Taiwan*

CO-AUTHORS: *Hao-Jan Liang, Sheng-Han Lee, Wen-Ling Chen, Tsun-Jen Cheng, Ching-Yu Lin*

Fine particulate matter (PM2.5) is able to pass the respiratory barrier and further enter the circulatory system, and consequently spread to the whole body. PM2.5-induced toxicity has been correlated with oxidative stress, which may lead to lipid perturbation. Our previous studies have applied a lipidomic platform to investigate the effects of PM2.5 exposure on the pulmonary lipids in rats inhaled ambient air, and found significantly altered phosphatidylcholine (PC) levels might impair pulmonary surfactant functions. However, the lipid effects of PM2.5 on other organs have not been fully elucidated yet. In this study, we examined the lipid effects of chronic PM2.5 exposure on various organs using a rat inhalation model. Five male rats were continually whole-body exposed to ambient air containing PM2.5 from the outside of the Public Health building in Taipei city for 8 months, while five rats were inhaled filtered air. Blood samples and various tissues, including heart, liver, kidney, pancreas, spleen, testis and epididymis were collected. Then the phosphorylcholine-containing lipids from each organ were extracted for further ultra-performance liquid chromatography system coupled with triple quadrupole mass spectrometry (UPLC-MS/MS) analysis and partial least squares discriminant analysis (PLS-DA). In our PLS-DA models, the patterns of phosphorylcholine-containing lipid were altered in the testis, pancreas, heart, liver and kidney of rats exposed to PM2.5. Numerous phosphorylcholine-containing lipid species associated with exposure were identified. Results also showed decreased PC(16:0/18:1) was both observed in the serum and testis. Further studies to verify potential biomarkers for PM2.5 exposure are needed.

P-310 Metabolomics-based biomarker discovery for bee health monitoring: A proof of concept study concerning diagnosis of nutritional stress in Bombus terrestris

PRESENTING AUTHOR: *Luoluo Wang, Ghent University, Belgium*

CO-AUTHORS: *Ivan Meeus, Caroline Rombouts, Lieven Van Meulebroek, Lynn Vanhaecke, Guy Smagghe*

Bee pollinators are exposed to multiple natural and anthropogenic stressors. Understanding the effects of a single stressor in the complex environmental context of antagonistic/synergistic interactions, is critical to pollinator monitoring and may serve as early warning system before a pollination crisis. This study aimed at methodically improving the diagnosis of bee stressors using a simultaneous untargeted and targeted metabolomics-based approach. Analysis of 84 Bombus terrestris hemolymph samples selected 8 metabolites retained as potential biomarkers that showed excellent discrimination for nutritional stress. In parallel, 8 significantly altered metabolites, as revealed by targeted profiling, were also assigned as candidate biomarkers. Furthermore, machine learning algorithms were applied on the above-described two biomarker sets, whereby the untargeted eight components showed the best classification performance with sensitivity and specificity up to 99 and 100%, respectively. Besides, based on pathway and biochemistry analysis, we propose that gluconeogenesis contributed significantly to blood sugar stability in bumblebees maintained on a low carbohydrate diet. Taken together, this study demonstrates that metabolomics-based biomarker discovery holds promising potential for improving bee health monitoring and to identify stressor related to energy intake and other environmental stressors.

P-311 iNVERTOX: Characterising background metabolomic variability and subsequent alteration in the freshwater invertebrate, Gammarus pulex, upon exposure to psychoactive pharmaceuticals

PRESENTING AUTHOR: *Thomas Miller, King's College London, United Kingdom*

CO-AUTHORS: *James I. MacRae, Nicolas R. Bury, Stewart F. Owen, Leon P. Barron*

The (pseudo)persistence of emerging contaminants in the environment represents a risk for the organisms that are exposed to them. 'Omics technologies are providing a powerful tool within environmental toxicology to better understand the effects of these exposure scenarios. However, interpretation of metabolite data to understand toxicological responses is challenging. The variability in individual metabolomes for a species, or a "background metabolome" should be established to determine possible confounding factors such as age, sex and moulting (among others) that may influence data interpretation. Thus, we have characterised the effect of these factors on the metabolic variability in the freshwater invertebrate, G. pulex. Herein, an analytical method is presented for the extraction and non-target analysis of the metabolome in G. pulex. Briefly, a dual phase liquid extraction was used followed by HILIC-HRMS to enable detection of metabolic features extracted from individual animals. Animals collected from the field were analysed immediately and compared to animals that were extracted after a fixed period of acclimatisation to laboratory conditions or exposure to environmentally relevant concentrations of antidepressants. The results indicated that biological sex, moulting stage and acclimatisation period affected the metabolic variability and should be investigated to aid understanding of pathways involved in effect-based studies. Furthermore, it may be prudent to pre-select animals based on these factors to reduce inherent variability in the data. Overall, the characterisation of metabolic variance for invertebrates along with the use of metabolomics shows a very powerful approach for understanding adverse effects that may be associated with environmental contaminants.

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P-312 Characterization of aerosol deposition on human organotypic respiratory epithelial tissue exposed at the air-liquid interface and metabolism assessment

PRESENTING AUTHOR: ***Sandra Sendyk, Associate Scientist, Switzerland***

CO-AUTHORS: ***Jeseena Sabaratnam, Shoaib Majeed, Laura Ortega Torres, Stefan Frentzel, Anita Iskandar, Julia Hoeng, Arno Knorr, Catherine Goujon***

Human organotypic respiratory epithelial tissue cultures are routinely used for in vitro toxicological assessment of biological impact upon exposure to cigarette smoke (CS) and aerosol from smoke-free products at the air-liquid interface. Metabolism of deposited compounds is a critical parameter to be considered for their bioavailability and assessment of potential toxicity. Nicotine is commonly used as a marker of exposure, and its metabolism pathway is well established. CS is a complex mixture of more than 6,000 compounds, a significant number of which have known toxicological effects and are associated with various smoking-related diseases. As CS or aerosol from smoke-free products represent a challenging matrix for in vitro testing and metabolite screening, a multi-compound method using dual-column liquid chromatography coupled to high-resolution accurate mass spectrometry was established. Nicotine and its major metabolites were quantified in extracts of organotypic respiratory epithelial tissue cultures following exposure to CS and aerosol from smoke-free products for 112 puffs. Quantification was performed by stable isotope-labeled internal standards for each analyte or analyte group. In total, three major metabolites of nicotine were identified, representing approximately 1% of the nicotine deposited on the investigated tissues. Additional exposure markers correlated with nicotine were investigated to better understand the deposition of aerosols and CS on organotypic respiratory epithelial tissue cultures.

P-313 Toxicity and metabolomics of zebrafish exposed to per- and polyfluorinated alkylated substances and alternatives to select more environmentally compounds

PRESENTING AUTHOR: ***Pim Leonards, Vrije Universiteit, Netherlands***

CO-AUTHORS: ***Jessica Legradi***

Per- and polyfluorinated alkylated substances (PFASs) have been widely used as organic surfactants in industrial and consumer products. While these applications made PFASs very appealing their properties make PFAS very persistent in the environment. In this study we performed a chemical alternatives assessment using metabolomics between PFOA and PFOS and alternative chemicals to be able to select environmentally friendly compounds. Zebrafish embryos (Danio rerio) were chronically and acutely exposured (0.1 µM , 1 µM , 10 µM) to PFOA and PFOs and its alternatives PFHxS, PFBS, siloxanes (D4, D5 and TMS), and GenX to study the mechanism of toxicity. No significant effects on mortality, visual developmental abnormalities, and swimming activity were observed for all compounds. Embryos were subjected to further investigation in an attempt to address potential genetic and metabolomic effects.Transcription of the DNA damage gene was significantly up-regulated by over 2-fold relative to the control vs. PFOA, PFHxS, PFOS and Genx. Interestingly, the lipid metabolism gene was up-regulated by over 2-fold in the embryos exposed to PFOS and GenX. These observations corresponds well with the metabolomics data that showed the regulation of many lipids (PC, PE, TAG, SM). Based on the results of this study, alterations in lipid metabolism (glycerophospholipids) may be a metabolic pathway affected by both PFASs and their alternatives. GENX, as alternative to PFOA, displayed a pattern of similar activity as the PFASs. The alternatives D4, D5 and TMS appears to have a lower impact on metabolic pathways (e.g. lipids) than PFOA.

P-314* Pre- and postnatal exposure to environmental pollutants alters lipid and polar metabolites profiles in non-obese diabetic mice

PRESENTING AUTHOR: ***Tim Sinioja, Örebro University, Sweden***

CO-AUTHORS: ***Johanna Bodin, Aidan McGlinchey, Daniel Duberg, Hubert Dirven, Hanne Friis Berntsen, Karin Zimmer, Unni Cecilie Nygaard, Matej Orešič, Tuulia Hyötyläinen***

According to the World Health Organization, chronic non-communicable diseases, such as heart disease, stroke and diabetes, are causing up to 60% of annual global mortality. The incidence of these diseases is not only due to genetic factors, but also to, or in combination with, the environmental factors, which comprise both external and internal exposures. External exposure, for instance to environmental contaminants, affects the internal exposure, i.e. biological factors, such as metabolism, which in turn mediate risks of various diseases. However, little is known how the exposure to chemical pollutants affects the metabolome. In this project, we explored the lipid and metabolic changes in mice following exposure to a mixture of persistent organic pollutants (POPs) containing organochlorides, organobromides, and per- and polyfluoroalkyl substances. Prior to blood collection, non-obese diabetic mice were pre- and postnatally exposed to two different concentration levels of POPs. Lipidomic profiling of mice blood serum samples was carried out on UPLC-Q-TOF/MS, while polar metabolite profiling was conducted on GC-Q-TOF/MS. Significant changes in lipid and polar metabolite profiles were observed between mice in the control and treatment groups. The largest difference in metabolite regulation was detected between control and the high exposure group. In particular, many phospholipids and several triglycerides containing polyunsaturated fatty acids were down regulated, while tricarboxylic acid cycle metabolites were upregulated. Our findings suggest that alteration of the lipid profile in mice exposed to the POP mixture is similar to previously reported metabolic changes associated with higher risk of type 1 diabetes.

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P-315 High-resolution Mass Spectrometry for Monitoring Physiological Impacts and Biotransformation Products in Fish Exposed to Wastewater Effluent

PRESENTING AUTHOR: ***Ioanna Ntai, Thermo Fisher Scientific, United States***

CO-AUTHORS: ***Jonathan Mosley, Marina Evich, Drew Ekman, Jenna Cavallin, Dan Villeneuve, Gerald Ankley, Tim Collette***

High-resolution mass spectrometry is advantageous for both metabolomics analyses and monitoring contaminant biotransformation products in fish exposed to wastewater effluent. For the current study, we conducted exposures of male and female fathead minnows to wastewater effluent using an onsite, flow-through system providing real-time exposure at the Western Lake Superior Sanitary District (WLSSD) wastewater treatment plant in Duluth, MN, USA. Metabolomic changes in liver tissue and skin mucus were measured for fish exposed for 21 days to control water or control water mixed with treated wastewater effluent at levels of 5%, 20% or 100% effluent. Skin mucus metabolomic changes reflected, in part, processes by which the fish biotransformed xenobiotics in the effluent. Regression models built with liver data provide additional evidence that xenobiotic transformation processes are induced, while also shedding light on the sex-specific nature of these responses. The detection of sex-specific phase II transformation products (e.g., glucuronidated bisphenol A) of chemicals from the effluent in the fish skin mucus led to the development of a novel untargeted method to identify glucuronidated biotransformation products in fish bile. Utilizing traditional enzyme hydrolysis, high-resolution mass spectrometry-based untargeted metabolomics methods, and a structure-specific neutral loss-dependent MS3 fragmentation method with a Thermo Scientific Orbitrap ID-X Tribrid mass spectrometer, we demonstrate this method using bile from these treated wastewater effluent-exposed fish as a potential means of screening for relevant contaminants found in complex environmental mixtures.

P-316 1H-NMR Metabolomics Study of Arabidopsis Thaliana Exposure to PFOA and PFOS

PRESENTING AUTHOR: ***Liam O'Hara, University of Guelph, Canada***

CO-AUTHORS: ***Julie Konzuk, James Longstaffe***

Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) are environmental contaminants originating from their use in industrial surfactants, textile finishes and flame retardants. Their widespread use has resulted in these compounds becoming ubiquitous environmental contaminants. The persistence of PFOA and PFOS poses an ecological and health risk due to movement up the food web through bioaccumulation. Non-targeted metabolomics provides an avenue to determine the subtle responses by plants when exposed to these compounds at environmental levels. This poster presents new work exploring the application of non-targeted metabolomics using nuclear magnetic resonance (NMR) to understand the effect of PFOA and PFOS contamination on plants. Arabidopsis thaliana was been chosen as the model organism, as previous studies have shown signs of oxidative stress at high concentrations of PFOA. Non-targeted metabolomics provided insight into changes expressed in the metabolism of the plants when exposed to environmentally relevant contamination. Principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) were used to determine the variation in the metabolic profiles in A. thaliana as a response to PFOA and PFOS contamination. This method could potentially be used on a wide range of species to determine whether they show signs of exposure to PFOA or PFOS contamination at levels that may otherwise be difficult to monitor.

P-317 Quantitation of glyphosate and AMPA from microbiome reactor fluids

PRESENTING AUTHOR: ***Beatrice Engelmann, Department of Molecular Systems Biology, Helmholtz Centre for Environmental Research-UFZ, Germany***

CO-AUTHORS: ***Katarina Fritz-Wallace, Stephanie S. Schäpe, Jannike L. Krause, Judith Pöppe, Gunda Herberth, Uwe Rösler, Nico Jehmlich, Martin v. Bergen, Ulrike Rolle-Kampczyk***

Glyphosate is one of the most widely used herbicides and potentially able to affect the intestinal microbiota, since it inhibits the aromatic amino acid synthesis via the shikimate pathways which is also used by bacteria. Chemostat cultivation of intestinal microbiota is increasingly used as a model system and consequently methods for quantitation of glyphosate and its degradation product AMPA in microbiome model systems are needed. Hence, an optimized protocol for the analysis of both glyphosate and AMPA by simple extraction with methanol:acetonitrile:water (2:3:1), while omitting further enrichment steps, was established. Glyphosate and AMPA were separated by hydrophilic interaction chromatography (HILIC) and identified with a targeted MS/MS method on a QTRAP 5500 system (AB Sciex). The limit of detection (LOD) in extracted water samples was < 2 ng/mL for both, glyphosate and AMPA. In complex intestinal medium the LOD was 2 ng/mL and 5 ng/mL for glyphosate and AMPA, respectively. The method was used to determine the glyphosate concentration in a bioreactor model of swine colon. Furthermore this method is compatible with the extraction protocol for an untargeted metabolomics analysis and feasible for different media used in microbiome reactors. The approach presented here allows the quantitation of glyphosate and AMPA in bioreactor fluids and thus enables studies on the metabolization of glyphosate and its impact on the intestinal microbiota.

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P-318* Metabolomics approach to study the environmental impact and residues of biocontrol products (BP)

PRESENTING AUTHOR: ***Mélina Ramos, PLS Université Paris: EPHE-UPVD-CNRS, USR 3278 CRIOBE, Université de Perpignan, France***
CO-AUTHORS: ***Marie-Virginie Salvia, Delphine Raviglione, Mercè Llugany, Esther Badosa, Emilio Montesinos, Cédric Bertrand***

Biopesticides or Biocontrol Products (BP) represents an interesting alternative to the conventional pesticides. However, there is a need of technical efficacy studies and ecotoxicological profile references. To date, the half-life, t1/2, was often used to study the fate of pesticides in environmental matrices. However, this value doesn't give any information regarding the formation of by-products and the effect on biodiversity. Consequently, an innovative approach based on metabolomics (LC-MS), the Environmental Metabolic Footprinting (EMF), was recently developed in the lab. On one hand, the EMF gives rise to a new integrative proxy, the resilience that corresponds to the time needed for the compound dissipation and its effects on the matrix (meta-metabolome). On the other hand, the EMF can be used in order to determine the preharvest interval (PHI) that corresponds to the time needed to have no residue (xenometabolome) difference between the treated sample and the control. The degradation of the BP on vine leaves was monitored. Vines were treated against powdery mildew with 2 fungicides BPs. The vine leaves were sampled at different time points between 2 treatments in order to study the BP degradation (kinetics). The extraction steps were optimized and the extracts were analyzed using a UHPLC-HRMS instrument. For now, only the xenometabolome was studied and the preliminary results showed a degradation kinetics for the 2 fungicides BP with a PHI between 4 and 10 days. The endometabolome is currently analyzed. The project lasts 3 years therefore the same study will be repeated and extended to other matrices.

P-319 Characterization of aerosol-derived water-insoluble organonitrates collected during wintertime at urban sites in China and Korea

PRESENTING AUTHOR: ***Kyoung-Soon Jang, Korea Basic Science Institute, South Korea***
CO-AUTHORS: ***Mira Choi, Minhan Park, A Young Choi, Moon Hee Park, Gun Wook Park, Young Hwan Kim, Yujue Wang, Min Hu, Kihong Park***

In this study, ambient fine particles (diameter less than 2.5 μm; PM2.5) were collected at urban cities in China and Korea (i.e., Beijing and Gwangju, respectively) during January 2018, and the dichloromethane (DCM) extracts of the PM2.5 samples were analyzed using an ultrahigh-resolution (UHR) Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer to identify the molecular characteristics of organic aerosols collected during wintertime at both urban cities. The FT-ICR MS analysis of the water-insoluble organic fractions (WIOCs) revealed that CHO and CHON class species were occupied more than 90% of the total WIOCs. The haze events with high organonitrate proportions were observed at both sites, but the CHON proportions at Beijing site have no correlation with secondary ionic species and gaseous components, implying that the nitrogen-containing organics originate from primary sources. We also found that the proportion of CHON class species at Gwangju site positively correlates with the concentrations of gaseous nitric acid and ammonia, indicating that ambient nitrate and ammonia has a role in the atmospheric formation of nitrogen-containing organic compounds at the Gwangju site. Particularly, significant proportions of CHN compounds were observed at Beijing site, while negligible amounts of CHN was detected at Gwangju site. Following two-dimensional gas chromatography time-of-flight mass spectrometry (GC GC-TOFMS) analysis identified the quinoline class compounds and the corresponding -CH2 homologous series from the WIOC at Beijing site. Interpretation of molecular changes of urban organic aerosols using an ultrahigh-resolution mass spectrometer and meteorological information could provide broad insight for understanding ambient organic aerosols.

P-320 Equine atypical myopathy: Targeted metabolomic study

PRESENTING AUTHOR: ***Radana Karlíková, IMTM, Palacky University, Olomouc, Czech Republic***
CO-AUTHORS: ***Petr Jahn, Jitka Široká, David Friedecký, Marek Mech, Lucie Mádrová, Hana Janečková, Františka Hrdinová, Tomáš Adam***

Equine multiple acyl-CoA dehydrogenase deficiency also known as atypical myopathy (AM), is a highly fatal muscle disease of grazing horses. This syndrome is accompanied by muscular weakness, acute myonecrosis and myoglobinuria, which in at least 75% of cases leads to death within 72 h. It is caused by ingestion of Acer Pseudoplatanus seeds containing hypoglycin A, whose active metabolite, the MCPA-CoA, is responsible for inhibition of FAD-dependent acyl-CoA dehydrogenases. The aim of this work was to compare the serum metabolomic profile of horses suffering from AM and controls and to confirm AM diagnosis in blood of newborn foal. Metabolomic analysis was performed using liquid chromatography with aminopropyl column coupled to tandem mass spectrometry (QTRAP 5500, AB Sciex). The metabolites were detected by multiple reaction monitoring in both positive and negative mode and statistically evaluated in R software. Analysis of specific biomarker MCPA-carnitine was performed by liquid chromatography-tandem mass spectrometry using the UltiMate 3000 system with BEH C18 column (50 mm, 1.7 μm, 2.1 mm) coupled to a triple-quadrupole mass spectrometer (Triple Quad 6500; SCIEX, Framingham, MA, USA). Significant differences were demonstrated in the concentrations of various glycine conjugates and acylcarnitines (C2–C26). Moreover, the concentrations of purine and pyrimidine metabolites, vitamins and selected organic and amino acids were altered in horses with AM. Metabolomic analysis of the foal's blood revealed increased concentrations of acylcarnitines and MCPA-carnitine consistent with metabolic profiles of blood from AM affected horses. The work was supported by the Czech Science Foundation Grant [18-12204S] and NPU I (LO1304)

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P-321 Optimization of comprehensive two-dimensional liquid chromatography (LCxLC) for the lipidomic analysis of zebrafish

PRESENTING AUTHOR: ***mengmeng xu, VU University, Netherlands***

CO-AUTHORS: ***Pim Leonards***

Background: Liquid chromatography (LC) coupled to mass spectrometry (MS) has become state of the art in lipid analysis. Reverse-phase (RP) LC, normal-phase (NP) LC, and hydrophilic interaction (HILIC) phases are often used. However, one-dimensional LC (1DLC) is not sufficient to cover the lipidome because of co-elution. Two-dimensional LC (2DLC) improves the separation power before detection with MS. Methods: The orthogonality of separation mechanisms is an important aspect in 2DLC (LC×LC). HILIC and RPLC were selected due to their solvent compatibility. A RPLC C18 column (2.1 mm × 100 mm, 2.6 µm) as the first dimension column, and a HILIC column (2.1 mm × 50 mm, 1.7 µm) as second dimension column were optimized for the separation of lipids. The combination of HILCI×RPLC was tested as well. A QTOF MS system was used for the detection of the lipids. Zebrafish eggs and larvae were analysed. Result: RPLC separated the lipids depending on the hydrophobic character and the lipids were separated based on the fatty acyl chain length and the number and positions of double bonds. In contrast, the separation of lipids by HILIC was based on the differences in the polar head groups of the specific lipid classes. The 2DLC analysis of zebrafish samples showed the separation of various classes of lipids (e.g. LPC, PC, PE, PI, PA, PS, DAG, TAG), and more than 1000 lipids were detected. The separation was much improved compared to 1DLC. Comprehensive 2DLC is a promising method for lipid characterizations of complex biological samples.

P-322 Insights into the toxicity of propazine on freshwater microalgae Chlorella Vulgaris (CV) and Scenedesmus Obliquus (SO): Untargeted Metabolomics via LC-QTOF

PRESENTING AUTHOR: ***Hiranya Dayal, National University of Singapore, Singapore***

CO-AUTHORS: ***Sam Li Fong Yau***

This study aims to utilize untargeted metabolomics using LC-QTOF to elucidate the impact of propazine, on freshwater microalgae. Freshwater microalgae are a group of phytoplanktons, popularly employed in biomonitoring and ecotoxicology studies. The toxicity of propazine was studied at a range of 10 µg/l -10 mg/l over 5 days on two freshwater microalgae, Chlorella vulgaris (CV) and Scenedesmus obliquus (SO). The growth rate of CV showed a gradual dose-dependent decline, while that of SO was significantly inhibited from 5 mg/l of propazine. Preliminary results reveal that in CV, the exposure resulted in a slow increase of monogalactosyldiacylglycerol (MDCG) and digalactosyldiacylglycerol (DDCG) levels with time, that may be attributed to enhanced membrane lipid synthesis. However, there was an observed decrease in MDCG and DDCG in SO from 100 µg/l that reached significance at 5 mg/l, which can be correlated with the observed growth inhibition at the same exposure level. Fluorescence-based assay for reactive oxygen species (ROS) further showed a gradual decrease in cellular ROS for CV with propazine exposure indicating that while growth rate of CV was slow with propazine exposure, some protection from further oxidative stress was provided with reinforcement of their cell membrane. Meanwhile, a significant increase in ROS was observed in SO from 5 mg/l propazine implying its limitations in tolerating environmental stress due to propazine. The results demonstrate that different microalgae have differing tolerances to propazine exposure, and reveal metabolomics as a tool to complement biochemical assays in assessing the environmental impact of herbicides on microalgae.

P-323 Framework for damage assessment of oryza sativa after chemical accident using metabolic effect level

PRESENTING AUTHOR: ***Woojung Kim, School of Earth Sciences and Environmental Engineering, Gwangju Institute of Science and Technology, South Korea***

CO-AUTHORS: ***Sangdon Kim***

As the changes in metabolome reflect mode of action specific cellular responses after chemical exposure, post-accident damage assessment based on metabolomics approaches emerged as a better alternative compared with physiologic approaches. The ministry of environment tried to characterize of plant damage by introducing metabolomics. However, target metabolite selection process was unclear and the exposure method did not reflect the chemical accident scenario, so the results had not been put to practical use. Thus, untargeted metabolomics and vapor exposure chamber were introduced in this study to overcome the limitations of existing research. Toluene was selected by taking into account both accident frequency and hazards. The oryza sativa was exposed in vapor exposure chamber. The metabolic responses of plants were analyzed by LC-QToF-MS based untargeted metabolic profiling and evaluated by multivariate statistical analysis and readable endpoints. The exposed concentration-based and recovery time-based metabolic response patterns were analyzed by principal component analysis and partial least squares discriminant analysis. In addition, the weight of shoot and root, the chlorophyll contents of leaves were assessed. Overall, the results of multivariate statistical analysis demonstrated a number of potential biomarkers that were characterized by metabolomic approach and provided an insight into quantitative chemical accident damage assessment.

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P-324 Metabolomics reveals kidney damage in mice exposed to thirdhand smoke

PRESENTING AUTHOR: *Carla Merino Ruiz, MIL@B - CIBERDEM - URV, Spain*

CO-AUTHORS: *Sara Samino, Beatrix Paton, Neema Adhami, Manuela Martins-Green, Xavier Correig, Noelia Ramírez*

Thirdhand smoke (THS) is a poorly understood pathway of tobacco exposure that is produced by the deposition and ageing of tobacco smoke particles and toxicants on surfaces, becoming progressively more toxic. Recent studies revealed the harmful health effects of THS exposure, but its effects on the kidney have not been studied yet. We applied untargeted metabolomics and lipidomics of the kidney and metabolomics of urine from mice exposed to THS under conditions that mimic the exposure in smokers' homes, using a multiplatform approach (UHPLC-QTOF and GC-QTOF). Kidneys from THS-exposed mice had significantly lower levels of betaine, which is a well-known kidney osmoprotectant. Additionally, different metabolites from pyrimidine metabolism and intermediates of alanine, aspartate and glutamate metabolism were also depleted in THS-exposed kidneys. Besides, several metabolites of the kynurenine pathway related to chronic kidney disease were found upregulated in urine of THS-exposed mice. Lipidomics analysis of kidneys showed that levels of different acylcarnitines, which together with carnitine are important in energy metabolism, and several lipids, such as glycerophosphocholines and sphingomyelins, were considerably lower in kidneys of THS exposed mice, leaving an impairment in energetic lipid metabolism. Interestingly, not all the metabolites altered as a result of THS exposure returned to near-control levels after an antioxidant treatment, indicating that the harmful effect of THS cannot easily be reverted. This study demonstrates for the first time that THS exposure is a latent risk to the development of kidney disease and, if confirmed in humans, would provide major evidence to enforce tobacco control policies.

P-325 Metabolic effects of a perinatal exposure to a low-dose pesticide cocktail in mice: sexual dimorphism and impact on the gut microbiota

PRESENTING AUTHOR: *Lorraine Smith, INRA Toxalim, France*

CO-AUTHORS: *Smith Lorraine, Klement Wendy, Barretto Sharon, Lasserre Frederic, Lukowicz Celine, De-Bock Frederic, Maillard Benjamen, Patris Bruno, Jamin Emilien, Debrauwer Laurent, Mselli-Lakhal Laila, Guillou Herve, Marchi Nicolas, Gamet-Payrastre Laurence, Ellero-Simatos Sandrine*

Epidemiological evidence suggests a link between pesticide exposure and development of metabolic diseases. We have shown a sexual dimorphic impact of a chronic dietary exposure to a low-dose cocktail of 6 pesticides (boscalid, captan, chlorpyrifos, thiofanate, thiacloprid, and ziram) on glucose homeostasis and gut microbiota in adult mice. Here, we aimed to investigate the consequences of a perinatal exposure to the same cocktail. Pesticides were incorporated in a standard chow at doses exposing mice to the tolerable daily intake of each pesticide. C57Bl6J female mice were exposed during gestation and lactation. After weaning, pups were fed a standard chow diet for 4 months, and then challenged or not with a high-fat for 2 months. We assessed metabolic parameters throughout the experiment. After 6 months, we evaluated host metabolism as well as gut microbiota metabolism using 1H-NMR-based metabolomics. Before the metabolic challenge, no difference was observed in metabolic parameters between exposed and non-exposed pups. However, the urinary and fecal metabolomic profiles were distinct and pesticide-induced perturbations were sex-specific. Trimethylamine, a host-gut microbiota cometabolite, was lower in urine of exposed, compared to unexposed males, while it was higher in urine of exposed females. Fecal metabolic profiles were also significant different in exposed vs. non-exposed males. After HFD, no difference was observed anymore in urinary metabolic profiles. However, fecal metabolic profiles were strongly altered in exposed vs. non-exposed females. Our results suggest that a perinatal exposure to pesticides could influence the gut microbiota metabolism with a significant diet-pesticide-sex interaction.

P-326 Advancing on the Chemical Characterization and Health Effects Associated to House Dust Exposure: Thirdhand Tobacco Smoke as Case Study

PRESENTING AUTHOR: *Noelia Ramirez, Institut d'Investigacio Sanitaria Pere Virgili-URV-CIBERDEM, Spain*

CO-AUTHORS: *Sonia Torres, Carla Merino, Beatrix Paton, Sara Samino, Neema Adhami, Manuela Martins-Green, Emma L. Schymanski, Xavier Correig*

House dust acts as a sink and a repository of toxicants, which persist during long periods of times or react with atmospheric oxidants to form secondary toxicants. Given people expend over 90% of their time indoors, a more accurate characterization of house dust chemical composition might provide valuable information about human exposome. A particular case of dust contamination is thirdhand tobacco smoke (THS), a novel and poorly understood pathway of tobacco exposure that is produced by the deposition and ageing of tobacco smoke particles and toxicants in surfaces and dust. This aged tobacco smoke becomes increasingly toxic with age, re-emitted into the air or react with other chemicals in the environment to yield new toxicants, including carcinogens. Although the increasing evidences of THS hazards, their accurate chemical characterization and the specific cellular and molecular consequences of THS exposure remain to be fully elucidated. We have analyzed smoker' and non-smokers' house dust samples using metabolomics-style non-target identification to identify masses with significant difference between the sample groups, followed by annotation using the in silico fragmenter MetFrag coupled to the CompTox Chemicals Dashboard and PubChem. Further, we have combined non-targeted metabolomics applied to two THS-exposed animal models (C57BL/6 mice exposed to THS under conditions that mimic exposure of humans in homes of smokers, and zebrafish embryos exposed to tobacco-specific nitrosamines), as well as, targeted analysis of neurotransmitters and specific biomarkers of tobacco exposure in children's urine exposed and non-exposed to THS. The results presented here provide key evidences about THS health hazards.

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P-327 Metabolomics in Industrial Research

PRESENTING AUTHOR: ***Regine Fuchs, BASF Metabolome Solutions GmbH, Germany***

CO-AUTHORS: ***Thomas Ehrhardt, Volker Haake, Tilmann Walk***

BASF Metabolome Solutions GmbH - formerly known as metanomics GmbH - provides mass spectrometry-based analytics, data interpretation and plant testing in the BASF Bioscience research platform since more than 20 years. With our competence in metabolomics, biological analytics and plant technologies we support innovation in agriculture, nutrition and industrial applications with mode of action analysis, biomarker identification and special metabolite analytics like volatile or wax analytics. Here we present how this integrated platform is applied for the toxicological evaluation of industrial chemicals.

P-329 Development of a food exposure and urine sampling strategy for dietary exposure biomarkers in free-living individuals using high resolution metabolite fingerprinting and targeted quantification

PRESENTING AUTHOR: ***Amanda Lloyd, Aberystwyth Univeristy, United Kingdom***

CO-AUTHORS: ***A.J. Lloyd, T. Wilson, N.D. Willis, L .Lyons, H. Phillips, L. Xie, E. Holmes, G. Frost, K. Tailliart, M. Beckmann, J.C. Mathers, J. Draper***

Dietary choices modulate the risk of chronic diseases and improving diet is a central component of public health strategies. Food-derived metabolites present in urine could provide objective biomarkers of dietary exposure when coupled with self-reported diet records. To assist biomarker validation we developed a food intervention strategy mimicking a typical diet with different food presentations, cooking methods and processing within complex meals and assessed urine sampling protocols potentially suitable for future deployment of biomarker technology. Six different menu plans representing comprehensively a typical diet that were split into two dietary experimental periods. Free-living adult participants (n=15 and n=36, respectively) were provided with all their food over a period of 3 consecutive days. Multiple spot urine samples were collected and stored at home. Flow Infusion-High Resolution Fingerprinting using Orbitrap MS coupled with High Performance Computer-enabled multivariate classification and feature selection were used to explore the composition of spot urine samples collected throughout the day. Biomarkers were validated using Ultra High Performance Liquid Chromatography quantification, using chemical standards. We successfully implemented a food exposure and urine sampling strategy for dietary exposure biomarker validation in free-living individuals. Biomarker performance was tested with different food formulations and processing methods involving meat, wholegrain and fruits/vegetables. Additionally, novel biomarkers were identified for foods where biomarkers have yet to be discovered in relation to the UK government healthy eating policies. Spot urine samples, together with robust dietary biomarkers that report multiple components of the UK diet should allow routine dietary exposure monitoring in large epidemiological studies.

P-330 Investigation of compounds responsible for the flavor of Peruvian chocolate from fine flavor cocoa

PRESENTING AUTHOR: ***Stephanie Michel, ICOBA - PUCP, Peru***

CO-AUTHORS: ***Alfredo Ibañez Gabilondo, Madina Mansurova***

Flavor is one of the most important characteristics of chocolate, besides being a key aspect to determine the price that the consumer is willing to pay. Currently, two types of cocoa beans have been characterized according to their profile of flavor and aroma, (1) the ordinary and (2) the fine flavor cocoa (FFC). In this project we have investigated how the flavor precursors presented in Peruvian FFC chocolate are transformed during each of the stages of chocolate manufacturing. We have identified eleven chemical compounds responsible for the fine flavor of Peruvian chocolate, and have analyzed their development during the main stages of chocolate manufacture using solid phase microextraction coupled to gas chromatography (HS-SPME-GC-MS) and direct analysis in real time (DART-MS). With this knowledge we can understand the chemistry of the Peruvian chocolate manufacturing, and establish a standardized manufacturing quality control protocol, that allows to local producers to improve the quality of their production.

P-331 High-efficient and High-throughput volatile flavor analysis of fermented seasonings using SA-SBSE

PRESENTING AUTHOR: ***YOKO IIJIMA, Kanagawa Institute of Technology, Japan***

CO-AUTHORS: ***Azusa Miwa, Hikaru Inagaki, Yusuke Ito, Kikuo Sasamoto, Takeharu Nakahara, Nobuo Ochiai***

In food metabolomics, specific food samples are frequently analyzed to determine the relationship between metabolome and sensory evaluation data, thereby establishing their flavor or nutritional properties, and optimal processing conditions. In Japan, fermented seasonings, such as soy sauce, miso paste and mirin (sweet rice wine), are very popular and have become particularly indispensable for the traditional Japanese cuisine. These seasonings have complex flavor properties, which are determined using several parameters derived from their materials, microorganism strains, fermentation conditions and maturation periods. Further, headspace analysis using direct extraction or solid-phase microextraction is a well-known, high-throughput, analytical method for volatile metabolomics. However, fermented seasonings contain several semipolar, odor-active volatiles that cannot be efficiently extracted from the headspace vapor phase, indicating that the volatile composition obtained from headspace extraction does not sufficiently reflect actual odor profiles. In the present study, we developed a new high-efficiency and high-throughput volatile flavor analysis using SA-SBSE (solvent-assisted stir bar sorptive extraction) for fermented seasonings. First, flavor volatiles were extracted from each sample using SAFE (solvent-assisted flavor evaporation), which is known as the most reliable flavor analysis extraction method. Odor-active volatiles were then screened using GC-Olfactometry. Next, we confirmed that SA-SBSE could extract the volatiles with high-efficiency, recovering >80% of the compounds detected using SAFE. Therefore, we suggest that SA-SBSE is suitable for flavor metabolomics of fermented seasonings owing to its extraction reproducibility and quantitativity.

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P-332 Discovery of Intake Biomarkers for Different Heat-Processed Potato Products by Metabolomics

PRESENTING AUTHOR: ***Xiaomin Zhou, University of Copenhagen, Denmark***

CO-AUTHORS: ***Cătălina S. Cuparencu, Natalia Vazquez Manjarrez, Gözde Gürdeniz, Lars Ove Dragsted***

Background: Potato is an important staple food in human nutrition, however, except for its contribution to energy and effects related to resistant starch the role of potato in human health is still debated. Biomarkers of food intake (BFIs) may be viewed as complementary tools for traditional dietary assessment tools. Therefore, we aimed to identify urinary exposure markers of potato products (cooked potato, chips and French fries). Methods: A randomized controlled single-blinded cross-over meal study was conducted. Four different intervention meals were prepared: boiled rice, boiled potato, chips and French fries. Discriminating features in urine were selected by the use of ANOVA and PLSDA as well as kinetics of excretion. Results: Thirty putative BFIs were found for heat-processed potato products which can differentiate between cooking and deep-cooking methods, two putative BFIs for potato in general and one for rice. Five of the 30 BFIs were identified or putatively annotated as conjugates of furaneol and 2,4-dihydroxy-2,5-dimethyl-3(2H)-furanone and hippuric acid. Among the rest were a range of conjugated compounds from Maillard reactions, and the proposed precursors were furans, pyrroles or pyridines. Conclusion: The study demonstrates that it is possible to find biomarkers for intake of potato products in urine samples. Combinations of identified or putatively annotated markers are proposed as potentially specific for intake of potato differentiated by different cooking processes. Perspective: Further studies are needed to identify the unknown biomarkers and to assess the robustness, reliability and repeatability in cross-sectional settings of the candidate heat-processed potato intake biomarkers identified in this study.

P-333 Metabolic profiling for utilization of soybean residues after protein extraction with different temperatures

PRESENTING AUTHOR: ***Hyejin Hyeon, Incheon National University, South Korea***

CO-AUTHORS: ***Cheol Woo Min, Sun Tae Kim, Jaeho Cha, Keumok Moon, Jae Kwang Kim***

Soybean is well known as one of the most important ingredients due to their enriched nutritional compounds such as functional peptides, carbohydrates, fatty acids and flavonoids. In order to use soybeans as principal sources of protein, novel aqueous processes for protein purification have been developed. However, these techniques have to be simultaneously concerned about the use of remained residues after protein extractions. The aim of this study is to reveal availability of soybean residues extracted at three different temperatures; 4°C, 25 °C and 55°C. Metabolic profiling with gas chromatography-mass spectrometry (GC-MS), GC-time of flight (TOF) MS and GC-flame ionization detector (FID) was conducted to investigate nutritional quality analysis of soybean residues. As a result, 64 metabolites including lipophilic compounds (policosanols, tocopherols and sterols), hydrophilic compounds (organic acids, amino acids, sugars and amine) and fatty acids were identified. Univariate and multivariate statistical analysis such as partial least-squares discriminate analysis (PLS-DA), hierarchical cluster analysis (HCA) and analysis of variance (ANOVA) showed that soybeans extracted at 55°C were notably distinguished with 4°C and 25°C. The significantly contributed metabolites for the discriminations were 8 amino acids, 7 organic acids, 3 sugars and 2 other compounds. This study represented that lots of essential and nonessential amino acids were accumulated by warm water extraction and galactose was interestingly increased by degradation of oligosaccharides known as anti-nutrients. Considering the nutritional effects of 55 °C extracted soybean residues, they expected to be used as feed for livestock to increase their market value.

P-334 Metabolite characterization for fermented rice koji by DOM (degree of milling)

PRESENTING AUTHOR: ***Sunmin Lee, Konkuk University, South Korea***

CO-AUTHORS: ***Da Eun Lee, Digar Singh, Choong Hwan Lee***

Koji, being an essential component or starter for numerous fermented foods and beverages, was utilized largely in food fermentative bioprocesses. A time-correlated metabolomic profiling was performed for fermented rice koji made using substrates with varying degree of milling (DOM). Overall, 54 primary and 13 secondary metabolites were selected significantly discriminant (VIP > 1, $p < 0.05$) among different koji samples by GC-MS and LC-MS. A higher abundance of carbohydrate (sugars, sugar alcohols, organic acids, phenolic acids) and lipid (fatty acids, lysophospholipids) metabolic intermediates with enhanced hydrolytic enzymes were observed for koji with rice's DOM 5–7. Conversely, functional metabolites such as flavonoids and phenolic acids, were relatively higher in koji with substrate's DOM 0, followed by DOM 5 > DOM 7 > DOM 9, 11, at 96 h. The grain milling significantly affected titratable acidity (DOM 0, 5 > DOM 7 > DOM 9, 11) and amino type nitrogen contents (DOM 5, 7 > DOM 0, 9, 11) in koji. In conclusion, the rice substrate preprocessing between DOM 5–7 was potentially ideal, with the end product being rich in distinctive nutritional and functional metabolites. The study rationalizes the grain preprocessing steps vital for commercial koji making.

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P-335 Mass Spectrometry-Based Untargeted Metabolomics and Alpha-glucosidase Inhibitory Activity of Reishi (Ganoderma lingzhi) during Growth Stages

PRESENTING AUTHOR: ***Dedi Satria, Kyushu University, Japan***

CO-AUTHORS: ***Dedi Satria, Sonam Tamrakar, Hiroto Suhara, Shuhei Kaneko, Kuniyoshi Shimizu***

Lingzhi (Chinese) or reishi (Japanese) is a Ganoderma mushroom species which has wide range of bioactivities. Analysis of metabolites changes during growth stages of lingzhi are important to understand underlying mechanism of its biosynthesis as well as bioactivity. It may provide valuable information for cultivation efficiency of lingzhi. In this study, mass spectrometry based untargeted metabolomics was carried out to analyze the alteration of metabolites during subsequent growth stages of lingzhi. Eight growth stages were categorized on the basis of morphological changes, starting from mycelium stage to post-mature stage of lingzhi. GC-MS and LC-MS analyses along with multivariate analysis of lingzhi growth stages were performed. Amino acids, organic acids, sugars, polyols, fatty acids, fatty alcohols, and some small polar metabolites were extracted as chemical markers from GC-MS analysis; while, lanostane-type triterpenoids were annotated from LC-MS analysis of lingzhi. The markers from untargeted analysis of lingzhi growth stages were correlated with alpha-glucosidase inhibitory activity. The current result shows that some metabolites are involved in the growth process and alpha-glucosidase inhibitory activity of lingzhi.

P-336 Comprehensive metabolomic analysis of sesame seeds

PRESENTING AUTHOR: ***Bo Mi Lee, Kookmin University, South Korea***

CO-AUTHORS: ***Eun Mi Lee, Byeung Kon Shin, Dong Jin Kang , Do Yup Lee***

Sesames are enriched with amino acid, essential fatty acids, carbohydrates, and hydroxy acid. The nutritional characteristics are mainly influenced by a range of environmental factors (e.g. climate and soil) as well as genetic factors. In current study, we analyzed the integrated metabolic profiles of sesame seeds grown in 29 Korean domestic provinces, 5 Chinese provinces, and 4 other countries (Nigeria, Ethiopia, India, and Pakistan). A total of 243 primary and secondary metabolites were acquired using gas-chromatography time-of-flight mass spectrometry (GC-TOF MS) and liquid-chromatography Orbitrap mass spectrometry (LC-Orbitrap MS). PLS-DA model primarily discriminated the sesames cultivated in Korea from Chinese one and those of 5 other countries. Based on the model, variable importance in projection (VIP) analysis prioritized 7 metabolites; glycerol, D-raffinose, DL-carnitine, histamine, 9-oxo-10(E), 12(E)-octadecadienoic acid, glycerophospho-N-palmitoyl ethanolamine, and palmitic acid. Receiver operating characteristic (ROC) analysis validated the discriminant power in area under the curve (AUC) ranged from 0.916 – 0.996. Particularly, the Korean sesames were best featured by higher abundances in a range of amino acids relative to Chinese and other countries' sesame. Further detailed interrogation was performed on 29 Korean sesames to examine if metabolomic profiles can distinguish the subtle regional differences. Hierarchical clustering analysis (HCA) proposed unbiased clusters of the sesames according to longitudinal coordination. The pathway overrepresentation analysis identified region-specific chemical categories as follows: aminoacyl-tRNA biosynthesis, galactose metabolism, alanine-aspartate-glutamate metabolism, and pentose-glucuronate interconversions. This study can be generalized under more controlled experiment, and applied to authentic decisions for cultivation origin of domestic agricultural products.

P-337 Pigmented sorghum polyphenols as potential inhibitors of amylolytic enzymes: An in vitro study combining starch digestion and untargeted metabolomics

PRESENTING AUTHOR: ***Gabriele Rocchetti, Università Cattolica del Sacro Cuore, Italy***

CO-AUTHORS: ***Gianluca Giuberti, Marco Trevisan, Luigi Lucini***

Polyphenols characterizing pigmented sorghum (PS) flours were in vitro evaluated as possible modulators of the activity of starch digestive enzymes. Flours from five PS varieties were analyzed after cooking and compared to white sorghum (WS) flour. An untargeted metabolomic approach comprehensively depicted the bound and free phenolic composition of each flour, highlighting a high distribution of flavonoids (i.e., anthocyanins, flavanol and flavone equivalents) and phenolic acids, with differences across samples. All PS flours were characterized by greater tannin and kafirin contents when compared to WS. Following in vitro digestion, all cooked PS flours showed greater resistant starch (RS) (from 4.2 to 21.4 g /100 g dry matter), as well as lower starch hydrolysis index (HI) when compared to WS. Multivariate statistics following untargeted metabolomics showed that anthocyanins and flavanol equivalents characterizing PS were the most discriminant compounds during the in vitro digestion. In addition, kafirin and total tannin contents (on raw ingredients) along with the anthocyanin profiles and the RS (on cooked samples) were negative correlated with HI. Therefore, PS flours might be useful in the formulation of foods characterized by likely functional properties.

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***AWARD WINNERS**

PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-338 Fiber mix supplementation in wheat-based flatbreads delays the exogenous appearance of glucose and its downstream metabolites

PRESENTING AUTHOR: ***Lisa Schlicker, Department for Bioinformatics and Biochemistry, BRICS, TU Braunschweig, Germany***

CO-AUTHORS: ***Hanny M. Boers, Christian-Alexander Dudek, Gang Zhao, Jean-Pierre Trezzi, Doris M. Jacobs, Karsten Hiller***

In nutritional intervention studies, the use of fully isotopically enriched plant material instead of single compound tracers enables deep insights into the postprandial catabolism of plant components (e.g. starch or protein). Due to its limited availability, only marginal amounts can be included in these studies, resulting in labeled plasma metabolites with 13C enrichment below natural enrichment of 1.1%, thus accurate quantification is analytically challenging. In a first step, we therefore developed an optimized mass spectrometry-based analytical workflow to achieve accurate quantification of 13C-enrichment in selected plasma metabolites below 1%. In a second step, we applied our workflow to plasma samples of a nutritional intervention study investigating the postprandial effects of fiber supplementation in wheat-based flatbreads. In this cross-over study, 12 healthy male subjects consumed flatbreads of different compositions: wheat flour (control) and wheat flour supplemented with 15% chickpea flour and different amounts of guar gum (2%: GG2, 4% GG4). We obtained time-resolved 13C enrichment profiles of 12 plasma metabolites namely glucose, lactate, alanine, glycine, serine, citrate, glutamate, glutamine, valine, isoleucine, tyrosine and threonine. While the postprandial metabolome was only mildly affected, fiber mix supplementation had a significant impact on the postprandial appearance of food-derived metabolites in the blood. Most interestingly, we found that fiber supplementation not only decreased the exogenous appearance of glucose. In addition, the appearance of the glucose-derived metabolites lactate and alanine was significantly reduced. The obtained data demonstrate the limitations of metabolite profiling and highlight that in vivo labeling contributes to a better understanding of nutritional interventions.

P-339 Untargeted metabolomics for the investigation of fish intake biomarkers

PRESENTING AUTHOR: ***Xiaofei Yin, University College Dublin, Ireland***

CO-AUTHORS: ***Helena Gibbons, Gary Frost, Lorraine Brennan***

Background: Fish intake has been reported to associate with certain health benefits; however accurate assessment of fish intake is still problematic. The objective of this study was to identify fish intake biomarkers and examine their potential for quantifying intake. Materials and methods: In the NutriTech study, ten participants were randomized into fish group and consumed increasing quantities (88 to 412 g/day) of fish for three days/week during three weeks. Fasting and postprandial urine samples were analyzed by 1H NMR spectroscopy and LC-MS. Dietary biomarkers were identified by multivariate data analysis and confirmed in National Adult Nutrition Survey (NANS) cohort. A calibration curve was developed and used to determine fish intake based on urinary biomarker levels. Results: Multivariate data analysis of fasting urine samples collected in NutriTech study revealed good discrimination between high (412 g/day) and low (88 g/day) fish intakes. Trimethylamine-N-oxide (TMAO), dimethylamine and dimethyl sulfone were identified and displayed significant dose response with fish intake ($P < 0.05$). Furthermore fish consumption yielded greater increase in urinary TMAO compared to red meat. In the NANS cohort, urinary TMAO levels were significantly higher in fish consumers compared to non-consumer ($P < 0.01$). However, the overall correlation between fish intake and TMAO (0.148, $P < 0.01$) were poor. LC-MS analysis revealed prominent increases in indoxyl sulfate and p-cresol sulfate excretion after high fish intake. Conclusion: Urinary TMAO displayed strong dose-response relationship with fish intake; however, use of TMAO alone is insufficient to determine fish intake in a free living population.

P-340 Screening of Biomarkers of Citrus unshiu Consumption Using Metabolomics

PRESENTING AUTHOR: ***Hisami Yamanaka-Okumura, Tokushima University, Japan***

CO-AUTHORS: ***Hisami Yamanaka-Okumura, Akiyoshi Hirayama, Aina Imai, Hiroshi Tatano, Daisuke Kajiura, Tomoyoshi Soga, Masaru Tomita***

Citrus unshiu is the most popular citrus in Japan. There are still no study biomarkers of the consumption of citrus using Citrus unshiu, necessitating their further screening. The present study aimed to investigate biomarkers and compare metabolites in plasma and urine following the consumption of several foods. The subjects were eight healthy men (age: 23.9 ± 1.1 years, BMI: 21.1 ± 2.6 kg/m^2) who participated in a randomized crossover trial. The test foods were apple, Cirus unshiu, broccoli, and seaweed (100g of each) that were consumed a single time a week. Metabolomic analysis was conducted on plasma samples from before consuming the test food and from 2 h after consumption and on urine samples from before consumption, 2 h and 4 h after consumption using CE-TOFMS. On analyzing the test foods, concentrations of Proline betaine were significantly higher in Citrus unshiu compared with concentrations following the consumption of the other foods in both of the plasma samples and urine samples. Of these, concentrations of Proline betaine and trans-Aconitate were significantly increased following the consumption of Citrus unshiu compared with those following the consumption of the other foods at 2h and 4h in urine. Candidate biomarkers of Citrus unshiu consumption were Proline betaine in plasma samples and urine samples. We showed that Proline betaine was a biomarker of Citrus.

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P-341 Identification of Flavonoid Metabolites in Urine and Plasma of Korean Fermented Soybean using an untargeted Metabolomic Approach

PRESENTING AUTHOR: ***Hwan-hee Jang, National Institute of Agricultural Sciences, South Korea***

CO-AUTHORS: ***Hwan-hee Jang, Heon-woong Kim, Hwayoung Noh, Su-yeon Cho, Hyeon-jeong Kim, Sung-Hyun Lee, Marc Gunter, Heinz Freisling, Jeong Sook Choe, Augustin Scalbert, Jung-bong Kim***

Fermentation may enhance nutritional and functional properties of food by increase of bioactivity or bioavailability during the process, which may have preventive effects on human disease. The study aimed to identify flavonoid metabolites of fermented soybean using an untargeted metabolomic approach and to investigate the effect of fermentation on bioavailability of flavonoids. In the cross-over intervention study with 10 study participants, standardized meals with fermented soybean (Cheonggukjang, fermented by Bacillus subtilis) and non-fermented soybean were served with a 1-week wash-out period in a randomized controlled trial. Flavonoids in each freeze-dried tested meal and flavonoid metabolites in 24-hour urine and blood samples collected before and after the consumption of each tested meal were measured by UPLC-DAD-QTOF/MS. Eighteen isoflavones, including genistein, daidzein, glycitein, and its glycosides, were identified in both tested meals. In the fermented food, levels of simple glycoside forms (e.g. daidzin and genistin) with high bioavailability were higher than those in the non-fermented food, which might be explained by the degradation of malonylglucosides during the fermentation of soybean. Fourteen metabolites including 7 glucuronides, 4 sulfates, and 3 sulfoglucuronides formed by conjugating enzymes were identified in urine and plasma, but equol and equol conjugated metabolites were not detected. The level of genistein 4',7-di-O-glucuronide was higher in the plasma collected after intake of fermented soybean when compared to non-fermented soybean. The results of the study may contribute to understanding the potential health effects of fermented foods by modifying the bioavailability and biological activity of soybean bioactive compounds.

P-342 Effects of organic acid salts and light treatment on policosanol concentration in Oat Sprouts (Avena sativa. L): development of a potent functional food

PRESENTING AUTHOR: ***Hyun Young Kim, National Institute of Crop Science, South Korea***

CO-AUTHORS: ***Hyeonmi Ham, Woo Duck Seo, Mi Ja Lee, Ji-Eun Ra, Hye-Young Seo, Kwang-Sik Lee, Ki-Chang Jang, Ki Do Park***

Elicitors are agents, such as chemical compounds, that trigger defense responses in plants, including the synthesis of secondary metabolites, such as policosanol. Policosanol consumption has various positive physiological effects in humans such as antioxidant capabilities, cholesterol-lowering activities, and improved liver function. Oat sprouts are a natural source of policosanol, making them a popular health food. The objective of this study was to test whether treatment of oat sprouts with organic acid salts and UV as an elicitor increases policosanol content compared with a control group of sprouts grown in water. During the growing phase, oat sprouts were treated with 0.1% sodium chloride (NaCl), 0.1% acetic acid, and UV360 bulbs at a wavelength of; samples were analyzed for policosanol content 10 days after germination using GC/MS. Results showed that 0.1% sodium chloride and 0.1% acetic acid treatments produced a policosanol concentration of 681.84 mg / 100 g and 736.4 mg / 100 g, respectively, compared with a water-grown control concentration of 554.8 mg / 100 g. The policosanol content in UV360-treated oat sprouts was also higher than that of the control sprouts at 651.4 mg / 100 g. In conclusion, cultivation of oat sprouts with organic acid salt supplementation and UV light treatment can significantly increase the levels of policosanol. This protocol could be used to develop a potent functional food with increased market value.

P-343 Spot and cumulative urine samples are suitable replacement sample types for 24-hour urine samples for objective measures of dietary exposure using metabolite biomarkers

PRESENTING AUTHOR: ***Thomas Wilson, IBERS, Aberystwyth University, United Kingdom***

CO-AUTHORS: ***Isabel Garcia-Perez, Joram M Posma, Amanda Lloyd, Edward S Chambers, Kathleen Tailliart, Hassan Zubair, Manfred Beckmann, John C Mathers, Elaine Holmes, Gary Frost, John Draper***

Self-reporting of dietary intake can be inaccurate and is prone to inherent bias. Measurement of multiple food intake biomarkers in urine may offer an objective method for monitoring dietary intake when used in conjunction with self-reported measures. In a randomised, controlled, crossover trial; participants four different diets (one per stay) over a 72 hour period, each with a stepwise variance in concordance with the World Health Organisation (WHO) healthy eating guidelines. Spot urine samples and cumulative urine samples were collected daily for the duration of the intervention. Urine samples were analysed using metabolite fingerprinting by both high-resolution flow infusion mass spectrometry (FIE-HRMS) and proton nuclear magnetic resonance spectroscopy (1H NMR). Absolute concentrations of selected dietary intake biomarkers were measured using liquid chromatography triple quadrupole mass spectrometry (LC-QqQ-MS) and by integration of 1 H-NMR data. Classification models for diet discrimination in spot, cumulative and 24-hour urine samples predicted dietary intake for all urine sampling time points. Cross validation modelling using 1H NMR and FIE-HRMS data also demonstrated the power of spot and cumulative urine samples in predicting dietary patterns in 24-hour urines. There was no significant loss of information when spot or cumulative samples were substituted for 24-hour urine samples. The combination of high dimensional classification modelling and quantitative analysis shows that when using urinary metabolic profiles and biomarkers for assessment of dietary patterns, spot samples are suitable replacements for 24-hour urine samples.

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PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-344 An integrated metabolomic and metagenomic analysis of Korean traditional alcoholic beverages using Nuruk

PRESENTING AUTHOR: *Jang Eun Lee, Korea Food Research Institute, South Korea*
CO-AUTHORS: *Jae Ho Kim*

Nuruk is a fermentation starter used for brewing alcoholic beverages. It is a kind of dough made from grains, such as wheat, barley, or rice, which are germinated by enzyme-releasing microorganisms. Since Nuruk mainly consists of starch, microorganisms that are capable of degrading β-starch are prominent in Nuruk. Specifically, various types of fungi, yeast and bacteria that cause alcoholic fermentation are present in Nuruk. Thus, it is important to understand how they affect the quality of the alcoholic beverages during fermentation. In this study, an integrated metabolomic and metagenomic approaches were used to generate the comprehensive metabolite and microbial profiles of Korean traditional alcoholic beverages produced by three different types of Nuruks selected from fifty-eight types of restored traditional Nuruk. Different analytical techniques such as GC-MS and 1H NMR in combination with multivariate analyses were used to obtain the metabolomic profile of each alcoholic beverage as well as that of the fermentation process. In addition, microbial communities were monitored to identify the correlative relationship between metabolites and microbes. The results indicated that the volatile metabolites of Nuruk-based alcoholic beverages are likely to be affected by microbial diversity in Nuruk, whereas the non-volatile metabolites are affected by the ingredients of Nuruk. These metabolomic profiles will be useful to identify how the metabolites and microorganisms of Nuruk affect the quality of alcoholic beverages. Furthermore, these results will be of great significance towards forming the basis of further research to scientifically establish the values of fermented foods.

P-345 Effects of estrogen deficiency on metabolic markers and gut microbiota in ovariectomized mice fed normal-fat diet

PRESENTING AUTHOR: *Youngmin Lee, Department of Food and Nutrition, Seoul Women's University, South Korea*
CO-AUTHORS: *Jieun Jeong, Chi Young Yun*

Estrogen deficiency is associated with obesity, dyslipidemia, and increased insulin resistance, yet underlying mechanisms remain poorly understood. This study was performed to investigate effects of estrogen deficiency on metabolic markers and gut microbiota in ovariectomized mice fed normal-fat diet. C57BL/6J mice (7 weeks old) were sham operated or ovariectomized and after 3 weeks, assigned to the following groups: sham-operated mice fed with normal fat diet (S-NF); ovariectomized mice fed with normal fat diet (OVX-NF). Experimental diets were fed for 5 weeks. At the end of the treatment, liver was isolated and serum was collected for biochemical analysis. Total DNA from fecal samples was extracted. Although there was no significant difference in food intake and weight gain due to ovariectomy in normal fat diet-fed mice, we observed a significant increase of liver weight and abdominal fat weight in OVX-NF group compared with S-NF group. No significant difference was observed in relative abundance of Bacteroidetes, Verrucomicrobia, Proteobacteria, and Deferribacteres among group. However, we observed that OVX-NF group had increased the phylum Actinobacteria, and decreased the phylum Firmicutes compare to S-NF group ($P<0.05$). In our results, there is a negative correlation between adiponectin level and Firmicutes, and a positive correlation between abdominal fat weight and Actinobacteria ($P<0.05$). This study suggested that changes in the gut microbiota may be related to estrogen deficiency-induced metabolic disorders in postmenopausal women. This study was carried out with the support of the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (NRF- 2017R1C1B5018328).

P-346 Targeted phenolic metabolites of purple potatoes in plasma and urine of healthy men

PRESENTING AUTHOR: *Johanna Jokioja, University of Turku, Finland*
CO-AUTHORS: *Johanna Jokioja, Kaisa M. Linderborg, Mark Philo, Jasmine Percival, Paul Kroon, Baoru Yang*

The purple hue of the coloured potatoes is derived from natural polyphenolic colorants called anthocyanins. Anthocyanins have a flavylium cation structure substituted with mono- or oligomeric sugars and in case of acylated anthocyanins, aliphatic or aromatic acids. Interestingly, anthocyanin-rich foods, such as berries, may be beneficial for health[1, 2]. Our recent study showed that purple potatoes may decrease human postprandial blood glycaemia compared to yellow potatoes [3]. The health effects of anthocyanin-rich foods may be contributed by their phenolic metabolites. However, the metabolic fate of dietary anthocyanins is still relatively unknown. Non-acylated anthocyanins are reported to be converted into glucuronide and sulphate conjugates and small phenolics[4]. The metabolites of acylated anthocyanins have been scarcely studied. Therefore, a cross-over clinical study was organized to investigate the phenolic metabolites in plasma and urine of healthy men after a meal rich in purple potato acylated anthocyanins. The samples were cleaned using solid-phase extraction, and the targeted metabolites were detected using mass spectrometry in multiple reaction monitoring mode. The anthocyanins were mainly of petunidin and peonidin glycosides acylated to hydroxycinnamic acids. Our results suggest that purple potato anthocyanins are metabolized into phenolic metabolites, such as phloroglucinaldehyde, hydroxybenzoic acids and hydroxycinnamic acids, and to their glucuronides and sulfates. [1] Li D et al (2015) J Nutr. 145, 742–748 [2] Törrönen R et al (2010) Br J Nutr. 103, 1094–1097 [3] Linderborg KM et al (2016) Int J Food Sci Nutr. 67, 581–591 [4] deFerrars RM et al (2014) Mol Nutr Food Res. 58, 490–502

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PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-347 Urinary food intake and microbiome-related biomarkers in lactating mothers and term and preterm infants

PRESENTING AUTHOR: ***Victoria Ramos-Garcia, IIS La Fe, Spain***

CO-AUTHORS: ***Jose David Piñeiro-Ramos, María Gormaz, Anna Parra-Llorca, Guillermo Quintás, Izaskun Garcia-Mantrana, María Carmen Collado, Máximo Vento, Julia Kuligowski***

The gut microbiota, host organism, and diet trialogue in lactating mothers and its impact on early nutrition programming of their infants is spotlighted in nutrition research. The analysis of the urinary metabolome provides a non-invasive means to determine a range of food intake and microbiome-specific biomarkers. In this study, liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) is proposed as a tool to study urinary metabolic biomarkers in lactating mothers and their term or preterm infants. Urine samples were collected from 27 lactating mothers (M), 29 term infants (TI) (25 receiving own mother's milk, OMM; 4 formula), and 47 preterm infants (PI) (20 receiving OMM, 20 donor human milk, DHM, and 7 formula). An untargeted LC-time-of-flight-MS assay was used for a comprehensive analysis of the urinary metabolome. Metabolite annotation and integration was performed matching against an in-house library containing 41 food intake and microbiome-related biomarkers described in the literature. Principal Component Analysis revealed characteristic patterns differentiating samples from M, PI, and TI. Employing Partial Least Squares Discriminant Analysis, significant differences between relative urinary biomarker concentrations of (i) M and TI, finding significant correlations for three food intake biomarkers, (ii) PI and TI and (iii) infants receiving OMM and formula were detected. No difference was observed between PI fed with OMM and DHM. The obtained results will be used for the development of a quantitative LC-MS-based method for studying the correlation between biomarkers and Food Frequency Questionnaires, as well as the composition of the gut microbiome.

P-348 Metabolomic Profiling of Superfood Seeds in bakery products using a Benchtop GC Time-of-Flight Mass Spectrometer

PRESENTING AUTHOR: ***Stefan Vinnenberg, LECO Instrumente GmbH, Germany***

CO-AUTHORS: ***Juergen Wendt***

Around 20 years ago "superfood" started to be used as a marketing tool for selling specific foods, dietary supplements and food additives. Although there is no exact definition of superfoods, one can be classified as food considered exceptionally good for one's health and for boosting the immune system. In the past few years chia seeds became one of the most popular superfoods. As a result of the rapidly increasing demand for superfoods, the market is flooded with counterfeits. In bakery products more expensive chia seeds are often replaced by cheaper flaxseed. The aim of this study was to evaluate the metabolomic profile of chia, flax and sesame seeds, looking for chemical markers, which allow confirming the presence of such seeds in food products. For sample preparation a well established derivatization protocol was applied, including a methoxymation step, followed by a silylation. The samples were analyzed using a LECO Benchtop GC-Time-of-Flight Mass Mass Spectrometer. A new TOF design has significantly improved the detection capability of the system, allowing for the safe identification and quantification of analytes, even at ultra-trace level. Different strategies for data evaluation were compared. Rosmarinic acid is best suited to distinguish between chia and other seeds. The marker was identified by the usage of the Golm Metabolome Database. By using this marker, alterations to bakery products can be easily uncovered.

P-349 Truffle aroma characterisation of Australia grown black Périgord truffle (Tuber melanosporum) during shelf life

PRESENTING AUTHOR: ***Maike Bollen, Metabolomics Australia, UWA, Australia***

CO-AUTHORS: ***Kenny Choo, Gary Dykes, Joshua Ravensdale, Ranil Coorey***

Truffles (Tuber melanosporum) are a high value commercial fungi traditionally produced in the northern hemisphere. The unique flavour and its rarity is due to the short growing season and relative difficulty for mass cultivation, making the truffle one of the most highly prized, edible fungi. Although truffles have been cultivated for decades, little is known of the ecology of truffle bacterial microbiome and the role it plays in the aroma development and spoilage during post-harvest storage. The main objective of this study was to determine the effects on the truffle aroma profile after chilled storage for extended periods of time using solid-phase micro extraction (SPME), together with gas chromatograph mass spectrometry (GCMS). Black Périgord truffle samples (N=14) were provided by Australian Truffle Traders from Manjimup, Western Australia, during the truffle season. The fresh truffle samples were assessed at three time intervals; 0, 7 and 14 days to monitor the changes in the metabolite profile of the truffles during storage. The results showed that 64 compounds were identified in all of the truffle samples at all time points. 11 key compounds showed significant changes between time points including dimethyl sulfoxide (DMSO) and 2,4-dithiapentane. In addition, dimethyl sulfoxide (DMSO) as well as 2,4-dithiapentane are compounds only found in white truffles (Tuber Magnatum). The findings of this study will provide truffle growers an insight on the impact of truffle aging on the aromatic qualities of truffles. Further studies are currently in progress to improve the preservation of the truffle aroma.

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PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-350 Exploratory analysis of commercial olive-based dietary supplements

PRESENTING AUTHOR: ***Mar Garcia-Aloy, Fondazione Edmund Mach, Italy***

CO-AUTHORS: ***Mar Garcia-Aloy, Nelli Groff, Domenico Masuero, Antonio Franco, Furio Battelini, Massimo Fia, Urska Vrhovsek, Fulvio Mattivi***

During the production of olive oil an important amount of wastes are generated. In last few years, their reutilization via recovery of polyphenolic fraction has attracted an increasing attention. Currently, the market of olive-based dietary supplements is composed of a broad range of natural extracts claiming different health effects and often sold without a clear statement on their chemical composition. The aim of the present study was to characterize the chemical profiles of a range of different olive-based dietary supplements. Such products were analyzed via untargeted metabolomics, using liquid chromatography coupled to high-resolution mass spectrometry (Orbitrap LTQ-XL), in order to explore their composition. PCA revealed a clear separation of samples according to the type of product (first component, 40% variance) and also according to the part of the olive-plant used (17% variance). The first component clearly divided gemmotherapy products from the other ones. Most of phenolics were present in these products in lower concentration (or not present). The second component was the one that separated the products according to the part of the olive plant used in their formulation, the fruits from the leaves. A Student's t-test was performed in order to select the putative biomarkers discriminating among the observed classes. Finally, most promising biomarkers were annotated, and the ones identified at level 1 were also quantified via HPLC Alliance DAD-QDa MS (Waters). The data produced with this study is suitable to support the unambiguous classification of the olive-based dietary supplements. Funding: Operational Plan "POFESR 2014/20" (Autonomous Province of Trento).

P-351 Food Intake Biomarkers for Increasing the Efficiency of Dietary Pattern Assessment through the Use of Metabolomics: Unforeseen Research Requirements for Addressing Current Gaps

PRESENTING AUTHOR: ***Cristina Andres-Lacueva, University of Barcelona, Spain***

CO-AUTHORS: ***Mar Garcia-Aloy, Raul González-Domínguez***

Current research on nutritional sciences depends upon the precise measurement of food intake. Despite being the most widely used dietary measurement tools, self-reported surveys are not exempt from already recognized limitations1,2,3. Low dietary assessment accuracy contributes to the inconsistency of results already observed in many instances when trying to understand the connections between diet and healthiness or disease risk, thereby weakening their potential translation to clinical and public health applications.2 The drawbacks of conventional instruments have encouraged research on food intake biomarkers (FIBs) as a complementary or alternative measure of dietary intake, being one of the cornerstones of nutritional epidemiology.2 Accurate dietary assessment is a challenge in nutritional research, needing powerful and robust tools for reliable measurement of food intake biomarkers. In this work, we have developed a novel quantitative dietary fingerprinting (QDF) approach, which enables for the first time the simultaneous quantitation of about 350 urinary food-derived metabolites, Thus, this metabolomic approach represents one-step further toward precision nutrition and the objective of improving the accurateness and comprehensiveness in the assessment of dietary patterns and lifestyles 1 J. Agric. Food Chem. 2018, 66, 5-7 2 Trends Food Sci. Technol. 2017, 69, 220–229. 3 Curr. Opin. Food Sci. 2017, 16, 96–994 4 J Agric Food Chem. 2019 Mar 1. doi: 10.1021/acs.jafc.8b07023 Funding: DiGuMet-PCIN-2017-076 CIBERFES 2017SGR1546 & ICREA Academia 2018

P-352 The effects of a brain-friendly diet on metabolic and physiological parameters and cognitive performance (Brave study)

PRESENTING AUTHOR: ***Johanna Koponen, Nightingale Health Ltd., Finland***

CO-AUTHORS: ***Marika Laaksonen, Sanna-Maria Hongisto, Juuso Parkkinen, Emmi Tikkanen, Juhani Sibakov, Jussi Loponen, Heli Diaz, Leila Fogelholm, Jukka Rantala, Harri Lindholm***

Cognitive performance is related to glucose metabolism and metabolic activation that are regulated by diet. We studied the effects of a brain-friendly diet (Brainfood) on metabolic and physiological parameters and cognitive performance in office workers at assumed metabolic risk.
We conducted a diet-switch, 4-week intervention study on 84 volunteers with elevated plasma LDL levels in pre-screening. The Brainfood diet focused on regular meal frequency and optimal intake of polyunsaturated fats, fibre and salt, whereas the control diet was a typical western diet. Plasma samples were collected at the end of the lead-in, control and intervention periods, and analysed using high-throughput NMR spectrometry for quantification of lipoprotein subclasses and particle sizes, cholesterol, glycerides, phospholipids, apolipoproteins, fatty acids, amino acids, ketone bodies, fluid balance, glycolysis and inflammation markers. A non-commercial mobile healthcare device recorded ECG, optical pulse plethysmography, accelerometric signal and thoracic impedance. Cognition was measured with standardized neuropsychological tests (Bourdon-Wiersma, FAT, N-Back, NASA-TLX, KSS, RAVLT, Stroop, WMS-III, Task-switching) under a psychologist's supervision and as tablet, PC or online tests. Brainfood reduced saturated fat and salt intakes and increased polyunsaturated fat, fibre, vitamin C and D, iron and magnesium intakes. Favourable effects were seen in the atherogenic lipid measurements, such as fatty acids, LDL, IDL and small VLDL, as well as fatty acids, phospholipids and glutamine. Intervention did not affect the physiological or cognitive parameters except for a decrease in inaccuracy of visual attention (p=0.027). The Brainfood diet could be recommended for office workers as a healthy diet following Nordic Nutrition recommendations.

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PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-353 Elucidating markers of strawberry consumption in smokers and non-smokers using metabolomics

PRESENTING AUTHOR: ***Jessica Cooperstone, The Ohio State University, United States***

CO-AUTHORS: ***Matthew D. Teegarden, Jennifer H. Ahn-Jarvis, Thomas J. Knobloch, Christopher M. Weghorst, Yael Vodovotz, Steven J. Schwartz, Jessica L. Cooperstone***

Background: Promising results from pre-clinical and clinical research support a potential role for berries and their associated phytochemicals in the prevention of oral cancer. Strawberries are a significant dietary source of berry phytochemicals. Our group recently conducted a randomized, cross-over, placebo-controlled clinical study, in which subjects consumed strawberries powder (24 g/day) and a placebo product, each for 7 days. Objective/Hypothesis: Our objective was to investigate key markers of strawberry exposure in the urine of healthy individuals and individuals at high risk for oral cancer (smokers) using metabolomics. Methods: Urine samples from 9 smokers and 10 non-smokers following placebo and strawberry interventions were volumetrically normalized according to osmolality and chemically profiled using UHPLC-QTOF-MS. Data were modeled using multilevel, multivariate analyses to capitalize on the cross-over design of the study. Results: A total of 41 markers were found, 26 of which were elevated following strawberry treatment and 15 following the placebo. Using ion fragmentation and comparison to authentic and synthesized standards, we identified metabolites elevated with the strawberry intervention including urolithin A (and phase II metabolites), dihydroxybenzoic acids, and phase II metabolites of strawberry aroma compounds. Markers of placebo consumption included phase II metabolites of flavorants and a metabolite of colorants included in the formulation. Conclusions: Distinct metabolic profiles pertaining to strawberry and placebo treatment arms were observed and provide targets for validation. These findings will inform future clinical trials with strawberry products with regard to potential markers of exposure and implications of study design on metabolomic profiles.

P-354 Identifying Natural Compounds as Inhibitors of Quorum Sensing Transcriptional Regulator (SdiA) of Klebsiella pneumoniae through Modelling, Docking and Molecular Dynamics Simulation

PRESENTING AUTHOR: ***Mohamed Alajmi, King Saud University, Saudi Arabia***

CO-AUTHORS: ***MD Tabish Rehman***

Bacteria can communicate amongst themselves through cell-cell signalling system; quorum sensing (QS). Gram-negative bacteria detect N-acylhomoserine lactones (AHLs) signalling molecules through LuxR transcriptional regulators. Certain bacteria do not produce signalling molecules but can detect the signals produced by other bacterial species through SdiA (suppressor of cell division inhibition). SdiA is a homolog of LuxR transcription regulator and thus regulates AHL-mediated transcription of various virulent genes. In the present work, we employed a computational biology approach to identify novel inhibitors of SdiA by screening natural compounds. First, we modelled the structure of SdiA using Modeller using 4LFU as template (64.6% sequence identity and 100% coverage). ProCheck, Verify3D, Ramachandran plot scores (>99.5% residues in the favourable and allowed regions) and ProSA-Web (Z-score=-8.45) have been employed to ascertain the good quality of the generated SdiA model. RMSD between SdiA model and 4LFU template was estimated to be 0.21 Å. The secondary structural contents of SdiA model was predicted using PDBsum. Further, CASTp identified only one pocket as the most probable binding site of SdiA (area=523.083 Å2 and volume=351.044 Å3). Autodock Vina was employed to perform high throughput virtual screening (HTVS) on a library of natural compounds available at InterBioScreen Ltd. The potential inhibitors were again docked on SdiA using flexible docking in Autodock. Further, molecular mechanics-general born surface area (MM-GBSA) was used to evaluate the effect of solvent on protein-ligand complex stability. Finally, molecular dynamics simulation was performed on the two best inhibitors to confirm the potential of the identified inhibitors against SdiA

P-355 Unraveling dynamic metabolomes underlying different maturation stages of berries harvested from Panax ginseng

PRESENTING AUTHOR: ***Mee Youn Lee, Konkuk University, South Korea***

CO-AUTHORS: ***Mee Youn Lee, Han Sol Seo, Digar Singh, Sang Jun Lee, Choong Hwan Lee***

Ginseng berries (GBs) show temporal metabolic variations among different maturation stages, determining their organoleptic and functional properties. We analyzed metabolic variations concomitant to five different maturation stages of GBs including immature green (IG), mature green (MG), partially red (PR), fully red (FR), and overmature red (OR) using mass spectrometry (MS)–based metabolomic profiling and multivariate analyses. The PLS-DA score plot based on GC–MS datasets highlighted metabolic disparity between preharvest (IG and MG) and harvest/postharvest (PR, FR, and OR) GB extracts along PLS1 (34.9%) with MG distinctly segregated across PLS2 (18.2%). Forty-three significantly discriminant primary metabolites were identified encompassing five developmental stages (VIP > 1.0, $p < 0.05$). Among them, most amino acids, organic acids, 5-C sugars, ethanolamines, purines, and palmitic acid were detected in preharvest GB extracts, whereas 6-C sugars, phenolic acid, and oleamide levels were distinctly higher during later maturation stages. Similarly, the partial least squares discriminant analysis based on LC–MS datasets displayed preharvest and harvest/postharvest stages clustered across PLS1 (11.1 %); however, MG and PR were separated from IG, FR, and OR along PLS2 (5.6 %). Overall, 24 secondary metabolites were observed significantly discriminant (VIP > 1.0, $p < 0.05$), with most displaying higher relative abundance during preharvest stages excluding ginsenosides Rg1 and Re. Furthermore, we observed strong positive correlations between total flavonoid and phenolic metabolite contents in GB extracts and antioxidant activity. Comprehending the dynamic metabolic variations associated with GB maturation stages rationalize their optimal harvest time per se the related agroeconomic traits.

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PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-356 Development of biomarkers of macronutrient intake by integrated analysis of metabolome data in both human samples and food compositions

PRESENTING AUTHOR: ***Yoshihito Fujita, Department of Diabetes, Endocrinology and Nutrition, Kyoto University, Japan***

CO-AUTHORS: ***Nozomu Sakurai, Kaori Ikeda, Nozomi Isomura, Fumika Mano, Futoshi Furuya, Daisuke Shibata, Takeshi Ara, Hiroto Minamino, Tsuyoshi Goto, Yasuki Matsumura, Teruo Kawada, Nobuya Inagaki***

Environmental factors such as excessive dietary intake have a critical role in the pathogenesis of life style-related diseases including diabetes and obesity; nutrition education contributes to the prevention of these diseases. Novel biomarkers of dietary intake are required to apply nutrition education effectively. To identify biomarkers of macronutrient intake, we have used a metabolomics approach. As a pilot study, we performed metabolomic analysis using human samples after test meal (Terumeal(R)). After intake of the test meal, increases and/or decreases in peaks detected by LC/MS analysis were distinguished in human plasma and urine samples. Correlation analysis of the metabolomic data of the test meal itself and the human samples revealed that many components derived from Terumeal(R) could be detected, and that the values were altered time-dependently after meal in human samples. Based on findings from this study, we established a protocol for the development of biomarkers of macronutrient intake. Next, we performed metabolomic analysis using human samples after white rice or white bread intake as representatives of carbohydrate, a component of macronutrient. Samples in 8 healthy volunteers were taken 7 times over a period of 24 hours. Increases in peaks by LC/MS analysis were detected after bread intake but were not detected after rice intake in human plasma samples. Components derived from bread or its derivatives modified in the body were detected in human plasma samples after bread intake. Thus, integrated analysis of metabolome data both in human samples and various food compositions revealed candidates for biomarkers of macronutrient intake.

P-357 Using different untargeted GC-MS trapping techniques reveals the comprehensiveness of volatile compounds in flavouring ingredients

PRESENTING AUTHOR: ***Carmen Diez Simon, Wageningen UR, Netherlands***

CO-AUTHORS: ***Roland Mumm, Robert D. Hall***

Metabolomics has become a valuable data-driven, holistic approach that is able to generate novel hypotheses, identify novel biomarkers and elucidate underlying modes of action. Whereas metabolomics has been applied to diverse plant materials, applications in processed foods is still in its infancy, especially in relation to flavour aspects. Nevertheless, recent studies have shown that metabolomics has great potential to identify sensory-relevant compounds. From this, it became evident that existing technologies have huge potential but need further development to obtain robust analytical data for complex food products. We have developed methods for the untargeted analysis of volatiles from process flavours. The challenge lies in developing optimal headspace trapping techniques that give the most comprehensive picture together with an efficient and robust detection of volatiles. A number of volatile trapping techniques including solid-phase microextraction (SPME), stir-bar sorptive extraction (SBSE), and dynamic headspace (DHS) were compared for their relative values and robustness for the analysis of the volatiles present. Results showed that the techniques differ in their efficiency in trapping of different classes of volatile compounds suggesting a need to tailor the volatile extraction technique depending on the specific composition of the process flavour. Methodologies have been developed which: deliver robust data on sensory-relevant compounds; allow for identifying unknown (Maillard-related) compounds that contribute to the prediction of sensory attributes and; give us deeper insights into the most relevant chemical reactions. Acknowledgements: The authors gratefully acknowledge funding from NWO, Unilever and DSM for the work presented.

P-358 Urinary Metabolomic Profiling to Identify Biomarkers of Carrot Intake

PRESENTING AUTHOR: ***Siva Charan Sri Harsha Pedapati, University College Dublin, Ireland***

CO-AUTHORS: ***Roshaida Abdul Wahab, Lorraine Brennan***

Metabolomics has emerged as a key technology to identify novel food-intake biomarkers in a range of foods. The objective of this research was to identify urinary biomarkers of carrot intake using an untargeted metabolomics approach. In this context, a randomized cross-over acute intervention study was conducted on eleven participants who consumed carrots (Test food) and turnips (control food) in random order. Urine samples were collected postprandially and analysed by UPLC-QTOF-MS. The data obtained was reduced to 3148 features in positive mode and 3015 features in negative mode applying filtering procedures such as data alignment and sample occurrence frequency. Robust PLS-DA models were obtained when comparing fasting samples with 12h (R2X=0.725, Q2=0.331) time point post-consumption of carrots. The variable importance for the projection (VIP) list was created from PLS-DA plots with scores ≥1.5 considered as discriminant features between the two time points. Time series plots revealed 29 features (15 in positive mode and 14 in -ve mode) with a time response following carrots consumption with 11 features demonstrating a differential time course compared to the control food. Ferulic acid 4-sulphate (m/z 273.0074), Pyrocatechol sulphate (m/z 188.9864), N-Feruloylglycine (m/z 251.0721), L-Tyrosine (m/z 182.0802) were identified as metabolites reflective of carrot intake using databases such as METLIN, HMDB and confirmed by LC-MS/MS. Future work will focus on confirmation of the remaining discriminating features and examine dose response relationship to ascertain biomarker validity. Overall, the untargeted metabolomics approach developed proved to be successful in the identification of urinary markers of carrot intake.

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PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-359 Australian native plants and their application in food

PRESENTING AUTHOR: ***Sara Ghorbani Gorji, School of Agriculture and Food Sciences, The University of Queensland, Australia***

CO-AUTHORS: ***Sara Ghorbani Gorji, Siyuan Chen, Zhenzhen Li, Melissa Fitzgerald***

Australia has a diverse native flora, that contains plants that have been used as food or medicine by Indigenous people for many thousands of years. However, to date Western knowledge about the properties of these plants is limited, and where information is available, it is clear that these plants offer unique properties in terms of flavour and nutritional value. Recently, there have been many reports about the nutritional value of Indian turmeric (Cucurma longa). In Northern Queensland, a native Australian turmeric (Cucurma australasica) is found, and it is reported that Indigenous people roast and consume the rhizomes. In this study we have explored the rhizomes of Australian native turmeric for the presence of phytochemicals important to human health, unique flavours, and nutritional properties, and compared these with the traditionally consumed Indian turmeric rhizomes. With the aid of metabolomics, the phytochemical, volatile compounds and minerals of Australian and Indian turmeric were studied. Various antioxidant assays were used to compare the antioxidant activity. The potential of using Australian turmeric in niche products such as turmeric latte or confectionary was also studied. Sensory analysis provided new insights about the application of Australian turmeric in the market. Our results showed that the Australian turmeric has comparable nutritional value to Indian Turmeric and furthermore, consumers preferred Australian turmeric over Indian turmeric in a formulation for turmeric latte. This research helps to understand potential applications of Australian native turmeric, ultimately increasing its economic value, and offering a native competitor to the now highly sought Indian turmeric.

P-360 UPLC-Orbitrap-MS and GC-TOF-MS based Metabolomics Analysis on the differentiation of Red Ginseng of Two Different Geographical Origins

PRESENTING AUTHOR: ***Ailsa Chui-Ying YUEN, The Hong Kong Polytechnic University, Hong Kong***

CO-AUTHORS: ***Siu-Wai WAN, Tung-Ting SHAM, Chi-On CHAN, Daniel Kam-Wah MOK***

Red ginseng (also called Hongshen in Chinese) is the steamed dried root and rhizome of Panax ginseng C.A. Mey and is a famous Chinese medicine for tonifying qi. The northern part of China and South Korea are two main cultivation areas of red ginseng, and ginseng produced in these locations differs in qualities and thus in their market prices too. Previous studies have used hydrogen, carbon and nitrogen isotopic ratios to differentiate ginseng collected from these two locations. However, this technique requires specialized instrument and does not provide information about the quality of red ginseng. Meanwhile, the analysis of ginseng mainly focuses on its ginsenoside content, however, it could be affected by various factors such as the steaming process, age and the part of the ginseng (main or lateral root). In this study, red ginseng samples collected from China and Korea were extracted using the modified Bligh and Dyer's liquid-liquid extraction method with subsequence analysis using GC-TOF-MS (aqueous layer) and UPLC-Orbitrap-MS (organic layer). Multivariate analysis was carried out to identify the key metabolites for differentiating between red ginseng from these two locations. Our results showed that carbodiimide, propionic acid, disaccharide (maltose, fructose, sucrose), ginsenoside Re, Rf, Notoginsenoside R2 could be tentatively identified as the metabolites to differentiate between ginseng from these two origins. The results suggested that the metabolomic profile of red ginseng could provide more comprehensive information to differentiate between Chinese and Korean red ginseng.

P-361 Metabolomics-based Approach for the Evaluation of Post-Harvest Quality of White Leg Shrimp (Litopenaeus vannamei) Cultured in Different Salinity

PRESENTING AUTHOR: ***Eiichiro Fukusaki, Osaka University, Japan***

CO-AUTHORS: ***Sri Hayati, Julie Ekasari, Sastia P. Putri***

Shrimp is one of the most important commodities in aquaculture industry and has important significance in global food supply. Salinity plays an essential role in shrimp aquaculture and is considered to affect post-harvest quality (physical and sensory attributes). However, there is no study on the correlation between salinity and metabolite profiles. The objective of this study is to investigate the effect of different salinity on physical and sensory attributes, and metabolome of white leg shrimp (Litopenaeus vannamei). As an initial analysis, shrimp was cultured in different salinity (10, 20, 30 ppt) for ten weeks under controlled condition and were subsequently analyzed for physical attributes (texture and color analysis), sensory analysis, and untargeted GC/MS analysis. The result for texture profile analysis showed that there is an increase trend in springiness and cohesiveness value along with increase of salinity level. Moreover, color measurement showed that ΔL*(lightness-darkness) and Δb*(yellow-blue) score showed a positive trend along with increase of salinity, and thus both of texture and color could be used as a reference for selected sensory attributes. GC/MS based metabolme data were subjected to Principle Component Analysis to clalify the important metabolites varying depending on salinity change to be several amino acids. The findings from this study provide an insight on the effect of different salinity towards metabolite composition of shrimp and can act as a useful feedback for shrimp aquaculture industry.

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PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-362 Discovering the flavor of the fine flavor cocoa of Piura/Peru

PRESENTING AUTHOR: ***Madina Mansurova, Pontificia Universidad Catolica del Peru, Peru***
CO-AUTHORS: ***Stephanie Michel, Alfredo J. Ibañez***

Flavor and aroma are the most important characteristics of chocolate and is key to determining the final price that the customer is willing to pay for the product. Peru is one of the countries that produces and exports the finest cocoa beans (Fine Flavor Cocoa, or FCC), and the chocolate as a final product. In this project we have developed an analytical method based on mass spectrometry to identify the volatile compounds profile responsible for the fine flavor of the Peruvian chocolate from Piura. Furthermore, we have observed the development of those taste markers during the manufacturing of the chocolate from cocoa beans. Finally, we cross-correlated the results of our measurements with the quality control team of the FCC-based chocolate company to corroborate with a panel the taste markers that we have detected molecularly. In summary, our methodology allowed us to map chemical compound patterns that represent the flavor and aroma of the final product (chocolate), making it possible to differentiate it from other ordinary cocoa chocolates, as well as from other fine flavor chocolate producers with different cacao origin or different manufacturing. Hence, this approach can be applied to other chocolate producers, as well as other food products to define, analyze and quantify flavor and aroma compounds. This information would be of great help for those companies whose sale price depends on their organoleptic characteristics.

P-363 Dynamics of metabolic profiling in thirteen pepper cultivars during fruit ripening

PRESENTING AUTHOR: ***Jae Kwang Kim, Incheon National University, South Korea***
CO-AUTHORS: ***Tae Jin Kim, Hyejin Hyeon, Nam Il Park, Tae Gyu Yi, Sang Un Park, Yongsoo Choi, Jae Kwang Kim***

To the best of our knowledge, none of the studies have conducted a comprehensive profiling of the primary and secondary metabolites in various phenotypes of pepper in relation to the stage of fruit maturity, nor did they have examined the metabolic networks by linking the large-scale metabolite data with metabolic pathways. We performed metabolic profiling of phytochemicals, namely amino acids, organic acids, sugars, sugar alcohols, free phenolic acids, capsaicinoids, carotenoids, phytosterols, policosanols, tocopherols, volatiles, and fatty acids, in 13 pepper cultivars at different fruit maturity stages. Our results comprised 10,218 data points resulting from three repetitions of 131 metabolites obtained from the fruit samples. Next, the multivariate analysis (principal component analysis, PCA; Pearson's correlation analysis; hierarchical clustering analysis, HCA) and PathVisio 3.3.0 were used with these data to obtain a more comprehensive understanding of the relationships among the metabolites and pepper samples. In the mevalonic acid pathway, the content of phytosterols such as campesterol, beta-sitosterol, cholesterol, and stigmasterol were reduced in the fruit mature stage, thus confirming the negative correlation between carotenoids and tocopherols revealed by the PCA and HCA. A complex carbon and nitrogen metabolic network was modulated by fruit ripening. Correlation-based network analysis suggested that metabolism is substantially coordinated during the ripening.

P-364 Regression model-based metabolomics approach to propose new quality marker for specialty coffee

PRESENTING AUTHOR: ***Sastia P. Putri, Osaka University, Japan***
CO-AUTHORS: ***Tomoya Irifune, Yusianto, Eiichiro Fukusaki***

Specialty coffee is defined based on cupping scores of 10 attributes (fragrance/aroma, flavor, aftertaste, acidity, body, uniformity, balance, clean cup, sweetness, overall) in the protocol established by the Specialty Coffee Association of America. Despite the increasing demand of specialty coffee, there are very few research on the relationship between component profile and specialty coffee quality. Such study is important for further quality improvement of coffee. Here, a regression model-based metabolomics approach was carried out to propose quality marker of specialty coffee. A total of 97 hydrophilic low molecular weight compounds were detected in 10 specialty coffee samples by GC/MS-based component profiling. Prediction models for 7 attributes (fragrance/aroma, flavor, aftertaste, acidity, body, balance, overall) and final score were constructed by Orthogonal Projection to Latent Structure regression analysis and these prediction models showed high performance (R2>0.9, Q2>0.9). Subsequently, important compounds for each attribute were proposed based on Variable Influence on Projection (VIP). This study proposed galactinol as a new candidate of quality marker of specialty coffee. Galactinol was shown as the compound with the highest VIP in the prediction models of fragrance/aroma, flavor, aftertaste, acidity and overall score. Quantitation of galactinol in samples with lowest and highest score samples in the prediction model showed that there is a significant increase of galactinol concentration in which highest scoring sample (cupping score 86.75) showed 8 times increase of galactinol concentration compared to lowest scoring sample (cupping score 80.25). Identification of galactinol as a new quality marker would be useful for quality improvement of coffee.

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PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-365 Metabolomics, an invaluable phenotyping tool in food science

PRESENTING AUTHOR: ***Melissa FItzgerald, University of Queensland, Australia***

CO-AUTHORS: ***Venea Dara Daygon, Sara Ghorbani Gorji, Crystal Concepcion, Lourdes Urban Andalete***

The field of metabolomics developed over the years in chemistry laboratories. However, over the past few years, the tools have become more available for routine use for phenotyping. In addition, the stability and reproducibility of the tools enables the phenotyping datasets to be associated with other datasets collected with other omics data, such as lipidomics and genomics. This enables us to complete the picture from the gene, its expression into a protein and the phenotype produced by the action of that protein. As we develop new ideas to improve the flavour, taste and nutritional value of food, metabolomics and other omics platforms have provided invaluable data for transformational solutions to the food industry. Metabolomics has enabled the discovery of different genes to improve the aroma of rice, different ways to provide natural ingredients to prolong the shelf-life of food, knowledge of how lipids impact the sensory properties of rice, and different ways to define rancidity based on volatile compounds. We will report our discoveries for different foods, using mostly metabolomics, lipidomics and genomics platforms, and demonstrate the value of these discoveries to agriculture and food industries in Australia.

P-366 Discovery of plasma biomarkers in human samples that reflect carbohydrate intake

PRESENTING AUTHOR: ***Nozomi Isomura, Department of Diabetes, Endocrinology and Nutrition, Kyoto University, Japan***

CO-AUTHORS: ***Yoshihito Fujita, Nozomu Sakurai, Kaori Ikeda, Fumika Mano, Futoshi Furuya , Daisuke Shibata, Takeshi Ara, Hiroto Minamino, Tsuyoshi Goto, Yasuki Matsumura, Teruo Kawada, Nobuya Inagaki***

Excessive carbohydrate intake is known as a major risk factor of lifestyle-related diseases such as diabetes. Clinical application of biomarkers that reflect dietary intake is required for nutrition education. In this study, we screened for biomarkers reflecting carbohydrate intake using a metabolomics approach. One-week consecutive intake of white bread followed by similar intake of white rice for the same period was applied for 7 healthy volunteers. Plasma samples were collected before and after each intervention, and non-targeted analysis using LC/MS was performed. In partial least squares (PLS) analysis, we discriminated 4 groups, which revealed dynamic changes of metabolites reflecting white bread or rice intake. To further investigate the biomarkers reflecting white bread intake, we compared PLS analyses to compare samples after the periods of bread intake or rice intake. We screened for peaks in mass chromatogram that reflected white bread intake specifically. Next, to ascertain whether these metabolites changed time-dependently, we performed analysis using samples after single intake of white bread or white rice. Plasma samples were collected 7 times over a period of 24 hours, and we found metabolites identical to those after 1-week intake. It is likely that these include external metabolites derived from the food composition itself. Thus, we found candidates for biomarkers that reflect carbohydrate intake such as white bread.

P-367 Compositional assessment of genetically modified soybean containing thioredoxin-encoding gene

PRESENTING AUTHOR: ***Ye Jin Kim, Incheon national university, South Korea***

CO-AUTHORS: ***Young Jin Park, Ju-Seok Seo, Jung-Ho Park, Chang-Gi Kim, Jae Kwang Kim, Sung-Dug Oh***

The development and cultivation of genetically modified organism (GMO) has been increased continuously over recent years. As cosmetic materials, genetically modified (GM) soybean event CT-4025 was developed. This soybean contains gene that encode thioredoxin (TRX) gene for improving skin whitening function. In addition, the phosphinothricin-N-acetyltransferase gene was used as a selectable marker gene for glufosinate tolerance. However, commercialization of the GM soybean requires the safety assessment. Therefore, in this study, to assess compositional equivalence between GM soybeans (non-sprayed and sprayed with glufosinate) and non-transgenic soybeans, forty-six key nutrients (proximates, amino acids, fatty acids, isoflavones, vitamins and anti-nutrients) were analyzed. The soybeans were cultivated in 2017 at two representative regions (Ochang and Jeonju) located in the Republic of Korea. Statistical analysis was performed by using the linear model and principal component analysis (PCA). As a result, most of the analyzed components in the GM soybeans had non-significant differences compared with its non-transgenic soybean in the linear model. A result of the PCA model showed that there was a clear discrimination between two regions rather than the soybean genotypes. In a loading plot, the isoflavones such as malonyldaidzin, malonylglycitin, glycitin and genistin mainly contributed to separation between Ochang and Jeonju. Consequently, these comparisons supported that CT-4025 is compositionally equivalent to the non-transgenic soybeans. Furthermore, the results of this study could be a useful tool to investigate compositional similarities between GM and non-GM food.

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P-368 Differences in the metabolomic profile and microbial composition of soil supporting Burkea africana growth

PRESENTING AUTHOR: ***Lufuno Ethel Nemadodzi, University of South Africa, South Africa***

CO-AUTHORS: ***Jacques Vervoort, Gerhard Prinsloo***

Burkea africana (Wild syringa) is a leguminous tree widely used for medicinal purposes, with a distribution in Namibia, Nigeria, Ethiopia as well as South Africa. The trees are found and grow in clusters, on dry, acidic sandy soils impoverished from most nutrients with a particularly low phosphorus level. The study determined the factors responsible for successful growth in nature of the Burkea africana trees as all efforts for commercial production has been proven unsuccessful. An investigation was carried out to comprehend the metabolic profile, chemical composition and microbial composition of the soils where Burkea africana grows (Burkea soil) versus where it does not grow (non-Burkea soil). 1H-NMR metabolomic analysis showed different metabolites in the respective soils. Trehalose, betaine, choline-like and carnitine-like compound, were found to be in higher concentration in the Burkea soils, whereas, acetate, lactate and formate were found to be more prevalent in the non-Burkea soils. LC-MS analysis revealed the presence of numerous amino acids such as aspartic acid and glutamine to be higher in the Burkea soils. Deoxyribonucleic acid (DNA) was extracted from the soil and subsequently sequenced. A Basic Local Assignment Search Tool (BLAST) was used to analyze the microbial diversity (bacterial and fungal) and composition found in both soils, for a comprehensive understanding of the soil composition. Penicillum species was found to be prevalent in Burkea soils and was the main discriminant between the two soils. The variances in fungal composition suggests that species supremacy play a role in development of Burkea africana trees.

P-369 Profiling Glucosinolates Using LC-MS in Seeds of Camelina sativa L. Crantz

PRESENTING AUTHOR: ***Rong Zhou, Agriculture and Agri-Food Canada, Canada***

CO-AUTHORS: ***Ning Xu, Kevin C. Falk, Margaret Y.Gruber***

Camelina sativa L. Crantz, a member of Brassicaceae family, is heat and drought tolerant. Thus it is thought to require fewer agronomic inputs and is a good alternative crop for marginal lands. Its seed contains three glucosinolates: glucoarabin (9-(methylsulfinyl)nonylglucosinolate or GS9), glucocamelinin (10-(methylsulfinyl)decylglucosinolate or GS10) and 11-(methylsulfinyl)undecylglucosinolate or GS11). The structures of these glucosinolates are similar to that of glucoraphanin (4-(methylsulfinyl)-butylglucosinolate). The degradation product of glucoraphanin is thought to be an anti-cancer compound. Glucosinolates were extracted and converted to desulfoglucosinolates based on AOCS Official Method Ak 1-92 before analyzed by a Waters Ultra Performance Liquid Chromatography-Photodiode Array Detector-Tandem Mass Spectrometry (UPLC-PDA-TQD) equipped with a BEH Shield RP18 column (2.1 x 50 mm; 1.7 µm) and held the initial conditions (100% water at 0.8 mL/min) for 0.3 minutes and then a linear solvent gradient of 0% to 25% acetonitrile (v/v) over the next 6.7 min. The desulfoglucosinolates were quantified at 229 nm and identified by monitoring the characteristic loss of 162.2 mass units using MS/MS constant neutral loss scans. UPLC reduced the typical LC run time of 25-50 minutes for camelina desulfoglucosinolates to 10 minutes and consumed 60% less acetonitrile. The camelina accessions surveyed contained 21 - 31 µmol glucosinolates per gram of seed: GS9 (4.6-7.0 µmol/g seeds), GS10 (13.7-20.2 µmol/g seeds) and GS11 (2.5-3.6 µmol/g seeds). Additionally, we identified several putative minor glucosinolates including 8-(methylsulfinyl)octyl glucosinlate (GS8) based on its mass spectrum. Further work is needed to confirm the identity of the minor glucosinolates.

P-370 Bitter Melon Triterpenoids Regulate Human Gut Microbiome and Potentially Benefit Metabolism Condition

PRESENTING AUTHOR: ***Jia Liu, Shanghai Institute of Materia Medica, China***

Bitter melon, a widely cultivated vegetable and medicinal herb from Cucurbitaceae family, has been used as a botanical dietary supplement in Asia for treatment of dyslipidemic conditions, diabetes, and obesity. The impact of bitter melon major components triterpenoids to gut microbiome was investigated. The results showed that bitter melon triterpenoids (BMTs), including tetracyclic triterpenes and pentacyclic triterpenes, could influence gut microbiota, but the effects varied for different volunteers. According to influence of microbiota, BMTs could be clustered. While some of them showed much stronger effect. Proportions of some beneficial bacteria were significantly promoted by BMTs; meanwhile, proportions of opportunistic pathogens were considerably decreased. Moreover, BMTs influenced the bile acids metabolism. After treatment, concentration of the TGR5 agonists and Farnesoid X Receptor antagonist bile acids increased, which indicated an increase of glucagon-like peptide-1 secretion. After component analysis of bitter melon from different regions, we found that proportions of pentacyclic triterpenes in bitter melons from Guangdong were significantly higher than that from Shandong and Hunan. Since different BMTs influenced microbiota condition and bile acid metabolism diversely, the influences of bitter melons with different chemotypes could vary. In conclusion, bitter melons could benefit to treatment of metabolic disorders through the influence to microbiota.

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PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-371 Metabolomic mapping of chemodiversity in microbial strains using NMR and multivariate statistical process control (MSPC) to find potential biomarkers

PRESENTING AUTHOR: ***John Gauvin, DSM, Netherlands***

CO-AUTHORS: ***Abel Folch Fortuny, Margriet Hendriks, Adriana Carvalho de Souza, Peter van de Vondervoort***

NMR has become an established technique for use in metabolic profiling, delivering information on a broad range of compound classes at various concentrations. In proton NMR many compounds give multiple signals within the proton spectrum, which can be used in statistical analysis to detect patterns in multivariate approaches. In a novel approach, we used the metabolic profiles of microbial strains to build a MSPC-based model to find possible biomarkers from the chemodiversity portrayed in spectra. MSPC has been used within industrial processes with many complex parameters and strongly multivariate data, to detect and diagnose deviations in incoming production batches. MSPC can be used to build a model from the "non-hits", considering them as the normal operating conditions results. Then, "hits" can be projected in the model and analyzed as outlier observations (since they were hits). Finally, in this work a MSPC-based model was created to map and distinguish biomarkers from general metabolites.

P-372 Metabolic engineering of Ustilago trichophora TZ1 for the improvement of malic acid production from glycerol using metabolomics approaches

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CO-AUTHORS: ***An N.T. Phan, Lisa Prigolovkin, Lars M. Blank***

U. trichophora has attracted increasing attention due to their capability in using glycerol, the main waste streams of biodiesel production process, to produce malic acid. Unlike other commercial filamentous fungi that tend to form pellets during cultivation, Ustilaginaceae can stay haploid and non-filamentous, which are the vital features for industrial applications. Previous optimizations could achieve the strain U. trichophora TZ1, which has the highest reported malic acid titre for microbial production from glycerol. Though, the production yield was quite low. The main obstacle for further strain improvement is the lack of knowledge on U. trichophora metabolic network. In this study, we aim to decipher and reconstruct the metabolic network in U. trichophora TZ1 to improve malic acid production using GCMS-based metabolomics. Metabolites in central carbon metabolic pathways were identified and quantified during malic acid production phase. As a results, the main by-product formation pathways in malic acid production from glycerol were revealed. By using metabolic engineering and medium optimization, we could decrease the level of by-products and successfully improve malic acid production in U. trichophora TZ1.

P-373 LC/MS-based metabolomics analysis for the improvement of 1-butanol production in engineered cyanobacterial Synechococcus elongatus

PRESENTING AUTHOR: ***Artnice Fathima, Osaka University, Japan***

CO-AUTHORS: ***Artnice Mega, Derrick Chuang, Walter Laviña, James Liao, Sastia Putri, Eiichiro Fukusaki***

Sustainable 1-butanol production using photosynthetic organisms, such as cyanobacteria, has garnered interest among researchers. 1-Butanol is natively produced via CoA-dependent pathway in Clostridia species. Therefore, a cyanobacterial, Synechococcus elongatus strain capable of producing 1-butanol, named BUOHSE, was developed by introducing a modified CoA-dependent pathway. However, 1-butanol production in this strain is low compared to other model microorganisms. Metabolomics approach for strain improvement has been widely employed in various microorganisms, which enables rapid detection of important metabolites and rate-limiting steps in the production pathway. Thus, comparative metabolome analysis of cyanobacteria strains producing 1-butanol was performed to further improve 1-butanol titer. By using ion-pair reversed-phase LC/MS, the reaction from butanoyl-CoA to butanal, catalyzed by PduP enzyme, was considered as a rate-limiting step. Accordingly, a new strain DC7 with an improved activity of PduP was developed. This strain showed a 33% increase in 1-butanol titer and a decreased level of butanoyl-CoA, indicating that butanoyl-CoA to butanal reaction was improved. To understand the effect of PduP optimization, metabolome analysis of DC7 and BUOHSE was carried out. Results showed acetyl-CoA was highly accumulated in DC7 compared to BUOHSE. To utilize this enhanced level of acetyl-CoA for 1-butanol formation in DC7, acetyl-CoA carboxylase was overexpressed. The resulting strain DC11 was able to reach a production titer of 418.7 mg/L in 6 days, cutting production time in half. In conclusion, metabolomics-assisted strain improvement employed in this study demonstrated the utility of metabolomics to effectively find a rate-limiting step and target for improvement in a biosynthesis pathway.

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***AWARD WINNERS**

PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-374 GC-MS metabolic profiling of volatile compounds produced by Actinobacteria

PRESENTING AUTHOR: ***Lada Brazdova, Institute of Microbiology, Czech Republic***

CO-AUTHORS: ***Bara Seidlova, Zdenek Kamenik***

Actinobacteria, particularly the genera Streptomyces, Amycolatopsis, Rhodococcus, and others, are important producers of bioactive metabolites including antimicrobials, antitumor agents, immunosuppressants, or signaling molecules. However, the mainstream research tends to focus on compounds, which are suitable for liquid chromatography, while volatile compounds may be missed unless they are analyzed by gas chromatography. We cultured various Actinobacteria strains with novel gene clusters including those encoding biosynthesis of new compounds with 4-alkyl-L-proline moieties. We determined the profile of volatile compounds by gas chromatography with mass spectrometry and processed the data using various approaches including GNPS molecular networking for GC-MS data.

P-375 LC-MS/MS and GC-MS metabolomics identifies feedstock components inhibitory to growth of engineered microbial strains

PRESENTING AUTHOR: ***Mona Elbadawi-Sidhu, Amyris, United States***

CO-AUTHORS: ***Chia-Wei Lu, Perry Kumagai, Quinn Mitrovich, Judith Denery***

Amyris strives to deliver high-quality, renewable products from sustainable resources, including cellulosic sugars. To achieve this, advanced microbial engineering coupled with state-of-the-art analytics are innovatively implemented to propel industrial synthetic biology and manufacturing of eight new renewable products in the past ten years. Targeted and untargeted mass spectrometry-based metabolomics promotes data-driven strain and process design that enables manufacturing of our products. One application of these approaches focused on development of strains capable of thriving on wood-derived cellulosic feedstocks. The conversion of wood into microbially-consumable monosaccharides produces a variety of side products that inhibit growth of highly engineered yeast strains. To better characterize the compounds affecting engineered strains, metabolomics was used to elucidate the complex composition of the feedstock. Polar and non-polar chromatography methods coupled to both LC-MS/MS and GC-MS were used to broaden the scope of the metabolic profiles. A multi-pronged approach was applied to determine the identities of the inhibitors: bioassay-guided fractionation of hydrolysate and spent hydrolysate informed the untargeted analysis, which in turn informed the targeted analysis, along with literature search of known feedstock components. Using untargeted metabolomics, we identified six novel components of cellulosic hydrolysate. In total, 70 compounds were tested, and we found that a combination of 26 metabolites could account for the total observed growth inhibition of the Amyris yeast. A summary of the analytical workflow and biological impact is presented.

P-377 Intracellular metabolite profiling and the optimization of metabolome extraction methods for Clostridium carboxidivorans fermenting syngas

PRESENTING AUTHOR: ***Yu Eun Cheong, Korea University, South Korea***

CO-AUTHORS: ***Jungyeon Kim, Kyoung Heon Kim***

Clostridium carboxidivorans, which ferments CO to biofuels via the Wood-Ljungdahl pathway, has received increased attention. Since CO is a unique substrate and is known to be metabolized via metabolisms different from other common carbon sources such as glucose, it is necessary to analyze intracellular metabolite profiles for CO fermentation by C. carboxidivorans to suggest clues for metabolic engineering. Moreover, when fermenting syngas, the metabolite profiles of C. carboxidivorans can be quite different from those under the normal conditions. Therefore, it is essential to optimize the metabolite extraction solvent for syngas-fermenting C. carboxidivorans. In comparison with glucose media, CO-containing syngas media allowed higher levels of intracellular fatty acid synthesis and changes of fatty acid metabolism possibly due to cofactor imbalance. The high levels of intracellular fatty acids could be used to produce high-value chemicals by further microbial engineering. Also, the evaluation of extraction solvents revealed that the mixture of water-isopropanol-methanol (2:2:5, v/v/v) is the best extraction solvent, which showed a higher extraction capability and reproducibility than pure methanol, the conventional extraction solvent for clostridia. This is the first metabolomic study to demonstrate the unique metabolite profiles of CO fermentation, and the mixture of water-isopropanol-methanol as the optimal metabolite extraction solvent for C. carboxidivorans fermenting CO.

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P-379 Development of LC-MS/MS based acyl-CoAs analysis method and its application to Streptomyces coelicolor

PRESENTING AUTHOR: ***Katsuaki Nitta, Osaka University, Japan***

CO-AUTHORS: ***Masahiro Furuno, Sastia Prama Putri, Eriko Takano, Eiichiro Fukusaki***

Acyl-CoAs are important intermediates in central carbon metabolism, fatty acids metabolism and other important pathways. In addition, secondary metabolism such as polyketide antibiotics synthesis also utilizes acyl-CoAs for intermediate elongation. Hence, a comprehensive analysis platform of acyl-CoAs is necessary to facilitate above pathway intermediate analysis. One of challenges of acyl-CoAs analysis is strong interaction of acyl-CoAs with metal part of LC-MS system and this results in strong peak tailing in liquid chromatography. Consequently this makes it impossible to detect low concentration of acyl-CoAs. In this study, the following five points were investigated to avoid the peak tailing: 1) metal free ODS column selection, 2) mobile phase pH and elution power selection, 3) chelating agent selection, 4) to inert ESI capillary, 5) to inert LC pomp and injector. In summary, non-metal column, which is resistant to highly basic mobile phase, was chosen for sustainable analysis while ammonium bicarbonate and acetonitrile was chosen as mobile phase to deprotonate the phosphate group of acyl-CoAs and avoid metal adsorption. To reduce metal part of LC-MS, fused silica capillary was employed as ESI capillary. In addition, bioinert pump and injector was employed. The peak shapes of acyl-CoAs were improved significantly in the optimized condition. In addition, different extraction solvents were investigated to achieve better coverage and mixture of acetonitrile, isopropanol & phosphate buffer showed good coverage. It was applied to Streptomyces coelicolor. A total twelve acyl-CoAs including highly hydrophobic(C16) acyl-CoA were detected and the LC-MS/MS analysis and extraction method showed good applicability to biological samples.

P-380 Evidence for Streptomyces venezuelae strains harboring potential anthelvencin biosynthetic gene cluster

PRESENTING AUTHOR: ***Mira Choi, Korea Basic Science Institute, South Korea***

CO-AUTHORS: ***Namil Lee, Woori Kim, Byung-Kwan Cho, Kyoung-Soon Jang***

Secondary metabolite biosynthetic gene clusters (BGCs), which encode bioactive compounds such as antibiotics and anti-cancer drugs, in Streptomyces species are often considered as a clue understanding the physiology and evolutionary aspect of those bacteria. In this study, we present highly resolved classification of 25 Streptomyces strains, representing ten S. venezuelae strains and other 15 species, using a matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometer and whole-genome mining. The close relationship of S. venezuelae ATCC 14583, 14584 and 14585 strains were revealed by comparison of the bacterial protein fingerprints by MALDI-TOF MS analysis, meanwhile those strains were not clustered by conventional 16S rRNA sequence alignment. Following whole-genome sequence alignment using 9 publicly available genome sequences of Streptomyces species and three S. venezuelae strains was used to evaluate their phylogenetic relationships, and the results correlate well with the MALDI-MS results. In addition, secondary metabolite BGC analysis of the genomes revealed that S. venezuelae ATCC 14583, 14584 and 14585 distinctly shared the congocidine BGC. Our findings in this study illustrate the importance of accurate bacterial classification and discovery of novel secondary BGCs for better utilization of metabolically relevant bacterial species.

P-381 The metabolisms in oxalate rich-leaf beetle via gut bacteria

PRESENTING AUTHOR: ***Atsuko Miyagi, Grad. Sch. of Sci. & Eng., Saitama Univ., Japan***

CO-AUTHORS: ***Noriyuki Ojima, Wakako Ikeda-Ohtsubo, Maki Kawai-Yamada***

Rumex obtusifolius is an invasive perennial weed, which is one of the most soluble oxalate-accumulated plant among Rumex species. Their oxalate accumulation is maintained at various constraints (high CO2 concentration, nutrition, dark, low temperature, and Al ion stress). Excess intake of soluble oxalate causes mineral (such as calcium and iron ions) deficiency or renal calculus for human and vertebrate. However, an oxalate rich-leaf beetle Gastrophysa atrocyanea feed R. obtusifolius as a host plant. To investigate the mechanism of oxalate metabolisms in the oxalate rich-leaf beetle, we performed metabolomic analysis of G. atrocyanea larva and adults using CE-MS. The results showed that oxalate was not accumulated in both larva and adults. Moreover, other organic acids such as lactate or citrate was highly accumulated. To clarify the effects of gut bacteria on oxalate degradation in leaf beetle, we measured the oxalate and other metabolites in the larva fed with an antibacterial agent tetracycline-treated R. obtusifolius leaves. The results showed that oxalate contents in the larva fed with tetracycline-leaves were highly accumulated than those in the larva fed with control leaves. Moreover, the tetracycline experiments revealed that the leaf beetle would obtain the gut bacteria from not their parents but their feeding leaves.

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***AWARD WINNERS**

PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-382 Contribution of gut microbiota to metabolism of dietary glycine betaine

PRESENTING AUTHOR: *Ville Koistinen, University of Eastern Finland, Finland*

CO-AUTHORS: *Olli Kärkkäinen, Klaudyna Borewicz, Iman Zarei, Jenna Jokkala, Valérie Micard, Natalia Rosa-Sibakov, Seppo Auriola, Anna-Marja Aura, Hauke Smidt, Kati Hanhineva*

The protective effect of whole grains against several chronic diseases is being supported by mounting evidence. At the same time, our knowledge is increasing on how gut microbiota influences our health and how diet can modify its composition. Herein, we studied C57BL/6J mice fed with bran-enriched diets, conventional and germ-free C57BL/6NTac mice, and the colonic fermentation of rye bran in an in vitro model of the human gastrointestinal system. We performed 16S rRNA gene sequencing and metabolomics to determine the effect of bran-enriched diets on the gut microbial composition and the potential contribution of microbiota to the metabolism of a novel group of betainized compounds. The bran-enriched diets elevated the levels of betainized compounds in the colon contents and changed the composition of the microbiota by increasing the relative abundance of several bacterial taxa, including Akkermansia, Bifidobacterium, Coriobacteriaceae, Lactobacillus, Parasutterella, and Ruminococcus. The levels of betainized compounds in the germ-free mice were significantly lower compared to conventional mice. In the in vitro model, the production of betainized compounds was observed throughout the incubation, while the levels of glycine betaine decreased. Only low levels or trace amounts of other betaines than glycine betaine were observed in whole-grain cereals. In conclusion, our findings provide evidence that the bacterial taxa increased in relative abundance by the bran-based diet are also involved in the metabolism of glycine betaine into other betainized compounds, introducing another compound group as potential mediators of the synergistic metabolic effect of diet and colonic microbiota.

P-383 Application of high-throughput MS screening to disentangle the bacterial metabolome

PRESENTING AUTHOR: *Riya C Menezes, Max Planck Institute for Chemical Ecology, Germany*

CO-AUTHORS: *Paolina Garbeva, Purva Kulkarni, Aleš Svatoš*

Bacteria produce a wealth of structurally diverse specialized metabolites with a remarkable range of activity. Recently augmented knowledge about bacterial genomes has revealed that many bacteria have far greater potential to produce secondary metabolites. This potential, however, has been hampered by difficulties in metabolite extraction or non-expression of genes. Many microbial gene clusters may be silent under standard laboratory growth conditions. One promising approach to trigger the activation of cryptic biosynthetic pathways is the co-cultivation of two or more microorganisms in the same confined environment, leading to the production of new metabolites. Because of the complexity of microbial extracts, advanced analytical methods are key for the successful detection and identification of co-culture-induced metabolites. In the present study, the secondary metabolites produced by 12 phylogenetically different bacteria isolated from soil and rhizosphere were analysed. Liquid Extraction Surface Analysis (LESA) combined with a Thermo Scientific LTQ Orbitrap XL mass spectrometer was applied using various solvents. The bacteria were also selectively cocultured and grown under three different nutrient conditions namely, nutrient-rich 1/10 TSBA; nutrient-poor Water Agar and Water Agar supplemented with Artificial Root Exudates. Our results revealed that LESA with high-resolution MS is a powerful tool for high-throughput extraction and detection of secondary metabolites produced by different bacteria under different culture conditions. Of all the solvents tested, ethyl acetate/acetone (65:35, v/v) containing 0.1% formic acid revealed the most effective extraction. Besides known secondary metabolites, several unknown metabolites were detected in bacterial cultures but not in any of the controls consisting of only media.

P-384 Mapping the chemical diversity between mammalian gut microbiomes – a molecular walk in the zoo

PRESENTING AUTHOR: *Rachel Gregor, Ben-Gurion University of the Negev, Israel*

CO-AUTHORS: *Rachel Gregor, Maraike Probst, Goor Sasson, Stav Eyal, Pieter C. Dorrestein, Michael M. Meijler, Itzhak Mizrahi*

In the past decade, studies on the mammalian microbiome have revealed that different animal species show wide variations in their microbial compositions. This diversity is strongly connected to a variety of factors, especially diet, digestive strategy, and animal host phylogeny. However, a key metagenomics study indicated functional redundancy, meaning that despite this taxonomic diversity, the functional capabilities of these microbial communities are similar, although certain functions were found to be enriched based on diet. Here, we propose to characterize the metabolic content of mammalian microbiomes as a direct window into ecosystem function. We have performed comprehensive metabolomics analyses on 101 mammalian fecal samples, as well as 34 dietary samples. Our platform includes LC-MS/MS for semi-polar metabolites and lipids, GC-MS for primary metabolites, and GC-FID for short chain fatty acids. Metabolites were identified using an in-house library of standards, and further explored using the Global Natural Product Social Molecular Networking platform (GNPS; gnps.ucsd.edu) to assign putative structures and group compounds into chemical families based on fragmentation patterns. Additionally, the microbiome of the fecal samples was analyzed by 16S rRNA gene sequencing. We found a strong correspondence between the animals' microbiomes and metabolomes (Mantel test: 0.703; significance: 0.001), as well the association of certain chemical families with different animal groups. We are currently examining how this effect differs for different classes of metabolites, as well as correlating between specific metabolites and microbial species, in order to better elucidate the metabolic capabilities of these communities and the ramifications on their ecological functions.

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***AWARD WINNERS**

PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-385 Mass spectrometry based quantification of intracellular metabolites

PRESENTING AUTHOR: ***Reza Maleki Seifar, DSM, Netherlands***

CO-AUTHORS: ***Reza Maleki Seifar, Lucien Duchateau, Maurien Olsthoorn***

Accurate quantitative data on intracellular metabolite from industrial cell cultures provides key information in metabolic engineering studies which is required for producing useful chemicals. In quantitative analysis of intracellular metabolites a number of important considerations regarding sampling and sample processing are need to be taken into the account: 1) short turn over time of some of the metabolites, 2) continuation of the metabolism of microorganisms at the time of sampling, 3) leakage of intracellular metabolites, 4) interferences from extracellular environments, 5) extraction efficiencies. Nowadays LC-ESI-MS(MS) is one of the most widely used analytical techniques in metabolomics studies. However, for absolute quantification of metabolites it is necessary to have access to standard chemicals and stable isotope labelled internal standards. Internal standards are required for correction of losses during extractions and also matrix effects in MS- based quantitative analysis of metabolites. The fully 13C-labelled internal standards could be considered as ideal internal standards. Here, we present the application of fully 13C-labelled internal standards which have been produced by microorganisms cultured on 13C-fully labelled glucose as exclusive carbon source. The sampling, quenching, extraction analytical method based on LC-MS/MS are presented for quantitative analysis of metabolites from two important mevalonate and isoprenoid pathways.

P-386 Evolution of gene knockout strains of E. coli reveal regulatory architectures governed by metabolism

PRESENTING AUTHOR: ***Douglas McCloskey, Biosustain, DTU, Denmark***

CO-AUTHORS: ***Sibei Xu, Troy E. Sandberg, Elizabeth Brunk, Ying Hefner, Richard Szubin, Adam M. Feist, Bernhard O. Palsson***

Biological regulatory network architectures are multi-scale in their function and can adaptively acquire new functions. Gene knockout (KO) experiments provide an established experimental approach not just for studying gene function, but also for unraveling regulatory networks in which a gene and its gene product are involved. Here we study the regulatory architecture of Escherichia coli K-12 MG1655 by applying adaptive laboratory evolution (ALE) to metabolic gene KO strains. Multi-omic analysis reveal a common overall schema describing the process of adaptation whereby perturbations in metabolite concentrations lead regulatory networks to produce suboptimal states, whose function is subsequently altered and re-optimized through acquisition of mutations during ALE. These results indicate that metabolite levels, through metabolite-transcription factor interactions, have a dominant role in determining the function of a multi-scale regulatory architecture that has been molded by evolution.

P-387 Metabolic profiling of Candida albicans

PRESENTING AUTHOR: ***Alberto Spisni, University of Parma, Italy***

CO-AUTHORS: ***Tecla Ciociola, Thelma A. Pertinhez, Laura Giovati, Walter Magliani, Alberto Spisni, Luciano Polonelli, Stefania Conti***

Microbial metabolomic analysis focused on extracellular and intracellular metabolites can be used for a number of purposes, including species identification. Currently, Saccharomyces cerevisiae Yeast Metabolome Database is the only one available of fungal metabolites. Here we outline the metabolic profile of Candida albicans SC5314 in different growth conditions. The metabolites of budding and germinating yeast cells were analysed by Proton Nuclear Magnetic Resonance (1H-NMR) spectroscopy. Yeast cells from semi-synchronized cultures suspended in fresh YPD and 199 medium were incubated at 30 and 37°C for 1 and 6 h. Extracellular metabolites, separated by centrifugation and ultrafiltration, and intracellular metabolites, obtained by mechanical cells disruption, were profiled by 1H-NMR on a JEOL 600 MHz ECZ600R and identified and quantified using Chenomx NMR suite 7.6. Distinct metabolic profiles are observed for cells grown in selected conditions. Increase in extracellular metabolites is observed over time (1 to 6 h). 50 intracellular metabolites were annotated. Among the major intracellular metabolites, ethanol, acetate, alanine, betaine, and pyruvate, metabolites involved in energy production pathways, were present in different concentrations in budding and germinating cells. Genes coding for enzymes involved in those metabolic pathways have been confirmed: Candida Genome Database (http://www.candidagenome.org). The results obtained, though preliminary, contribute to increase the knowledge of C. albicans metabolic profile and to create its metabolites database. This strategy will allow to unravel the pathways involved in the transition from budding to germinating phenotype. Characterization of C. albicans main metabolic pathways favour the identification of hot spots for new antifungal agents.

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***AWARD WINNERS**

PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-388 Exploring the Metabolic Fate of Dietary Polyphenols

PRESENTING AUTHOR: ***Nicola Gray, University of Reading, United Kingdom***

CO-AUTHORS: ***Cíntia Rabelo e Paiva Caria, Christine Hinz, Chris Titman, Wagner Vilegas, Glenn Gibson, Gunter Kuhnle***

Epidemiological and dietary intervention studies suggest that consumption of polyphenol-rich foods may reduce the risk of cardiovascular disease. Increased intake of polyphenols, found at high levels in tea, fruit and vegetables, has been associated with health-promoting benefits including vascular and platelet function, blood pressure and improved plasma lipid profile. However, the mechanisms behind these benefits remain unclear, and the bioavailability of dietary polyphenols is highly variable between individuals. A significant fraction of dietary polyphenols can persist in the colon, where they are exposed to the gut microbiota. Since the study of the human gut microbiota is hindered by the complexity of this ecosystem and accessibility, in vitro gut models provide a powerful tool to build mechanistic knowledge around microbial polyphenol bioconversion. Combined with metabolomics, this approach enables investigation of broad metabolite perturbations and variations in microbial diversity to enhance mechanistic understanding of polyphenol bioactivity and nutritional influences to improve health and reduce risk of CVD through diet. Here, untargeted and targeted LC-MS metabolite profiling was applied to in vitro gut models from human stool donors in the presence of different flavanol-containing substrates to explore polyphenol metabolism and the influence on gut microbial activity. Distinct metabolic differences were observed in the in vitro culture profiles with the addition of polyphenol substrates in a time dependent manner. Targeted phenolic, bile acid and short chain fatty acid profiles allowed for a more detailed exploration of gut microbial metabolism, providing an in-depth workflow to determine the bioconversion of dietary polyphenol compounds.

P-389 Semi-targeted analysis of folate metabolites in yeast using ultra-high resolution mass-spectrometry

PRESENTING AUTHOR: ***Lena Gmelch, Technical University of Munich, Germany***

CO-AUTHORS: ***B. Kanawati, P. Schmitt-Kopplin, M. Rychlik***

Folates are a group of vitamins consisting of more than 50 different vitamers. These vitamers play an important role in many metabolic processes such as methylation reactions, nucleotide synthesis, or oxidation and reduction processes of C1 units. A lack in folates is closely associated with the incidence of neural tube defects and is assumed to be a major risk factor for several chronic diseases such as cardio vascular diseases or dementia. Until now, folates are mainly analyzed in the respective monoglutamate forms. Little is known so far about the distribution of polyglutamates, which are the major vitamer forms in food. Thus, it is important to gain deeper insights into the metabolism of folates including its polyglutamylated forms. Therefore, a semi-targeted FT-ICR-MS approach was conducted to unravel the complete vitamer distribution including metabolic precursors. Baker's yeast served as model organism since it is known to be a rich source of folates. For obtaining higher sensitivity, sample clean-up via SPE had to be performed prior to measurements. Several signals could be unraveled as tentative folate vitamers. For verification of those compounds, targeted analysis by LC-MS/MS using a low resolution triple quadrupole detector was performed. The results were in good agreement with FT measurements and thus, the assignment could be confirmed. To further investigate the folate metabolism, yeast grown in different chemically defined culture media was analyzed as described above. Differences in the vitamer distribution as well as higher folate contents in media containing methionine could be observed as described previously.

P-390 Development of a Caenorhabditis elegans Reference Material for Long-Term LCMS Metabolomics Quality Control and Unknown Compound Identification

PRESENTING AUTHOR: ***GONCALO GOUVEIA, University of Georgia, United States***

CO-AUTHORS: ***David L. Blum, Alison Morse, Arthur S. Edison, Lauren M. McIntyre***

Robust quality assurance/control measures are an essential component of metabolomics studies. Creating a biological reference material (BRM) that is compliant with six-sigma best practices improves our ability to make inferences from metabolomics experiments. However, this is a challenging task. There are several criteria for a BRM: it should contain features that can be identified on different instruments and platforms and should be able to be produced over-time with negligible variation. This enables corrections for runs, instruments, and time. C. elegans cryo-resistance allows for their genetic identity to be preserved, making it an ideal BRM. Genetically identical batches can be produced from the identical genetic source over-time, a primary source of variation in biological systems. The other source of variation is in the environment during growth. To minimize environmental variance, small batches are produced in bioreactors allowing uniform growing conditions and a homogenous batch. The largest remaining source of variation is the food. We have successfully generated a stable C. elegans food source (E. coli) by ensuring that individual batches are homogenous, combined with an adaptation of methods originally developed by "Student" that include small-batch averaging. QC for individual batches and small batch averaging are common strategies in manufacturing that ensure long-term product consistency. We have divided the process into steps: environmental input control, batch QC, and batch averaging and developed QC metrics for batch acceptance or rejection before averaging. We evaluated our approach by measuring metabolites by NMR and our results indicate that that averaged batches can be 99.99% concordant.

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P-391 Metabolomes of Sander lucioperca and Rutilus rutilus lacustris gills and lenses

PRESENTING AUTHOR: ***Lyudmila Yanshole, International Tomography Center SB RAS; Novosibirsk State University, Russian Federation***
CO-AUTHORS: ***Ekaterina Zelentsova, Vadim Yanshole, Yuri Tsentalovich***

The metabolomic analysis of fish tissues can be successfully used for the study of impact of external factors on the fish health, including water contamination with pesticides, herbicides, low oxygen level. Changes in the metabolomic compositions of tissues reflect the health status, and metabolomic studies helps to elucidate the molecular basis of pathogenesis or the influence of ecological factors. For example, a problem of extremely high cataract prevalence in farmed salmonids leads to the increase in the metabolomic studies of fish lenses. Earlier we developed a method for quantitative metabolomic analysis of different tissues and biofluids. In this work we applied our approach to the analysis of metabolme of roach and pike-perch lenses and gills. The quantitative metabolomic profiling of samples has been performed with the combined use of high frequency 1H NMR and high-resolution LC–MS methods. The concentrations of 60-80 metabolites in fish lenses and gills have been measured. The most abundant metabolites in the fish lenses are N-acetyl-histidine, N-acetyl-aspartate, serine-phosphoethanolamine and threonine-phosphoethanolamine. These metabolites were attributed to osmolytes. Ovothiol A, one of the most powerful antioxidants existing in nature, was found in fish lenses in millimolar concentrations. The results obtained in the work show high dependence of osmolyte concentration on the season - N-acetyl-Histidine predominates in autumn fish lenses, while myo-inositol - in winter lenses. This difference may be attributed to the low oxygen level in water during the winter season and to the seasonal changes in fish nutrition. Supported by RFBR (Projects N° 19-04-00092, 18-29-13023).

P-392 Plasma metabolomic profiling in chickens under heat stress

PRESENTING AUTHOR: ***Shozo Tomonaga, Kyoto University, Japan***
CO-AUTHORS: ***Hirofumi Okuyama, Tetsuya Tachibana, Ryosuke Makino***

In the poultry industry, exposure to high ambient temperature induces harmful effects, such as growth retardation, increased mortality, and decreased quality and quantity of edible parts (meat and eggs). However, the precise mechanisms underlying physiological and pathophysiological changes induced by heat exposure remain unknown. To understand the influence from a metabolic perspective, we investigated the effects of exposure to high ambient temperature on plasma low-molecular-weight metabolite levels in chickens using gas chromatography/mass spectrometry-based metabolomic analysis. The heat exposure suppressed growth and food intake. Of the 92 metabolites identified, the levels of 29 decreased, whereas the levels of 9 increased. An enrichment analysis using the identified metabolites indicates 35 metabolic processes affected by heat exposure. Among them, the sulfur amino acid metabolic pathway was clearly detected. Changes in the kynurenine pathway in tryptophan metabolism, which could be linked to the immune system and oxidative stress, were also observed. Among the kynurenine pathway metabolites, we reconfirmed increase of quinolinic acid by the quantitative method. These results suggest the possible involvement of various metabolic processes in heat-exposed chickens. Some of them would be important to understand the mechanism of biological responses to high ambient temperature in chickens.

P-393 The search for novel anthelmintic targets: Characterizing alternative metabolic pathways in Caenorhabditis elegans

PRESENTING AUTHOR: ***Margot Lautens, University of Toronto, Canada***
CO-AUTHORS: ***Samantha Del Borrello, Kathleen Dolan, June Tan, Amy A. Caudy, Andy G. Fraser***

Parasitic helminths infect around a quarter of the human population and place a large economic burden on agricultural industries but anthelmintic resistance is a growing problem. Helminths survive long periods of hypoxia in their hosts, but their anaerobic metabolism has yet to be fully characterized. These anaerobic metabolic pathways are a promising target for new drugs. The goal of this project is to find novel, selective drug targets in parasitic helminths by characterizing alternative metabolic pathways in the free-living helminth model, Caenorhabditis elegans. Fumarate reduction, a rewiring of the TCA cycle, has been previously identified as a major metabolic pathway involved in the survival of C.elegans and parasitic helminths during hypoxia. It relies on rhodoquinone (RQ), absent in helminth hosts. RQ-deficient mutants are unable to recover from prolonged exposure to the Complex IV inhibitor cyanide (KCN). To test whether KCN induced fumarate reduction, the TCA cycle phenotype of RQ-deficient mutants and rotenone-induced Complex I disruption was assayed by liquid chromatography-mass spectrometry (LC-MS) and found to be similar. The metabolomic profiles of chemical and genetic disruption of fumarate reduction, knockout of the hypoxia transcription factor, hif-1, and recovery from KCN were interrogated by LC-MS and a computational pipeline developed to allow for metabolite hits to be easily called and visualized. So far, changes in vitamin and starch metabolism have been identified. These pathways will be chemically and/or genetically disrupted then subjected to an in-lab imaging assay to determine their importance to KCN recovery and thus their viability as drug targets.

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***AWARD WINNERS**

PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-394 Integration of metabolomic approaches in contemporary analysis of bioactive natural products

PRESENTING AUTHOR: ***Maria Halabalaki, National and Kapodistrian University of Athens, Greece***

Natural products (NPs) still retain a predominant position among chemical entities since they are characterized by an unmet and untapped structural diversity and uniqueness. This particular nature is the positive consequence of the evolutionary process, which furnished NPs with competitive advantages, enabling them to interact with biological targets for the benefit of every living organism. The NPs privileged features have led to their broad utilization in medicine, pharmacology, and biology but also in nutrition, agriculture, cosmetics, biotechnology, food chemistry, environmental chemistry, and botany, to name just a few. In the last years, NP's discovery process is experiencing a technological breakthrough. New approaches have been incorporated altering traditional concepts and perceptions. One of these approaches, which was rapidly embraced by the experts in the fiend of NPs, is metabolomics. NP metabolomics, have been rapidly employed for the study of natural entities in a more holistic way, finding multiple applications and uses. Thus, they have been incorporated for chemotaxonomic studies, safety and toxicity assessment, quality control, plant defense, exploration of physiological and biochemical effects in different environmental and stress conditions as well as for the investigation of the physiological development of different organisms and drug discovery. The integration and harmonization of metabolomics with traditional and contemporary methods and approaches effectively facilitates the NPs research. In the context of this talk, different applications of NP metabolomics will be presented using specific examples related to NP drug discovery, chemotaxonomy and quality control of natural entities such as medicinal plants and foodstuffs.

P-395 Metabolic implications of gut health disorders in Norwegian farmed salmons as studied by high resolution magic angle spinning (HR MAS) 1H-NMR spectroscopy of fecal samples

PRESENTING AUTHOR: ***Violetta Aru, Department of Science, University of Copenhagen, Denmark***

CO-AUTHORS: ***Bekzod Khakimov, Elvis Chikwati, Alexander Jaramillo Torres, Aleksei Krasnov, Trond Kortner, Paul Johan Midtlyng, Åshild Krogdahl, Søren Balling Engelsen***

The "GutMatters" project, funded by the Norwegian Seafood Research Fund (FHF), aims at defining the prevalence of gut health disorders and their incidence in sea farmed Atlantic salmon. The project plan included the sampling of a total of 360 salmons from 6 farm sites along the Norwegian coast and over one year period (3 time points). Histopathological analysis, performed on gut samples, revealed inflammatory changes predominantly in the distal intestine but also in the whole intestinal tract in fish with chronic cestode parasitosis. Additionally, enterocyte steatosis was observed in the pyloric caeca of individuals collected in most of the farm sites. HR-MAS (high resolution magic angle spinning) 1H-NMR (proton nuclear magnetic resonance) spectroscopy was used for the first time to analyze intact fecal samples collected from the pyloric caeca and distal intestine of Atlantic salmons. The samples were measured on a 400 MHz spectrometer. The results of the metabolomics analysis evidenced a similar metabolic composition for the samples from the two intestinal regions. Amino acids (i.e. alanine, methionine, and phenylalanine), organic acids (i.e. lactic acid) and sugars were found to be the main components of the fecal metabolome. Significant alterations in the lipid composition of the fecal samples were observed as a result of both dyslipidemia and gut inflammation.

P-396 Exploring the adaptation of rainbow trout metabolome to novel aquafeeds by 1H-NMR metabolomics

PRESENTING AUTHOR: ***Simon Roques, INRA, Univ. Pau & Pays Adour, E2S UPPA, UMR 1419 Nutrition Métabolisme et Aquaculture, France***

CO-AUTHORS: ***Catherine Deborde, Nadège Richard, Sandrine Skiba-Cassy, Annick Moing, Benoit Fauconneau***

The growing industry of aquaculture faces the limited availability of marine resources used to feed farmed fish. The development of plant-based diets alleviates this bottleneck, but carnivorous fish such as rainbow trout still do not tolerate diets based on plant ingredients only. Our objective was to explore fish responses to plant-based diets complemented with alternative raw materials: insect, microalgae, yeast or processed animal proteins. Metabolites were profiled in fish tissues and biofluids using NMR. We also analysed the composition of alternative raw materials to identify compounds susceptible to operate in fish metabolism. The relevance of metabolomics in fish nutrition is illustrated with three results. (1) Histidine, an essential amino acid, was negatively correlated in muscle and plasma with the specific growth rate whatever the diet. In fish, it is considered as a protective buffer agent and prevents the decrease of glycolytic flux during anaerobic process. (2) Betaine, glycine and serine signals were modified in the muscle of fish fed plant-based diet compared to a commercial one. This may correspond to a one-carbon metabolism alteration or to an accumulation from raw material compounds. (3) A quinone compound, specific of microalgae, could be used to assess their incorporation in diet: it was present in raw material and in the liver of fish fed microalgae. Such an approach opens new ways to integrate the interpretation of fish metabolism in diet effect studies. Funding: FUI 2014 (NINAQUA with Le Gouessant, COPALIS, Algae Natural Food, Phileo-Lesaffre Animal Care), ANRT (CIFRE 2016/0775) and MetaboHUB (ANR-11-INBS-0010).

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***AWARD WINNERS**

PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-398 Metabolomics, mixture models and maggots: solutions to agricultural waste problems and food security

PRESENTING AUTHOR: ***Elizabeth Dickinson, University of York, United Kingdom***

CO-AUTHORS: ***Mark Harrison, Marc Parker, Michael Dickinson, Guy Beavis, James Donarski, Maureen Wakefield, Rosie Nolan, Aida Rafat, Jason Hallet, Adrian Charlton, Julie Wilson***

Food security is a global challenge and industrial waste biomass is a major environmental problem. Palm oil waste is currently burned as a means of disposal, and solutions are required to reduce this industry's environmental impact. Palm waste can be recycled into biogas, however, we aimed to optimise conditions for recycling into a nutritious substrate for farmed insects. As a natural component of the diet of farmed animals such as fish, chicken and pigs, fly larvae could meet the increasing demand for protein in animal feed, and can be grown on plant waste. Palm waste is tough and needs to be broken down using microbes (anaerobic digestion), providing a substrate for larvae and producing methane gas, a sustainable energy source. However, digestion processes require adjustments and optimisation, therefore composition of the substrate was investigated. NMR spectroscopy and LC-HRMS were used to analyse palm empty fruit bunches (EFB) after various pre-processing methods, then after anaerobic digestion. Biogas production was also measured. Statistical pattern recognition techniques, such as principal component analysis (PCA), were used to investigate compositional differences before and after digestion, and partial least squares regression (PLS-R) was used to produce models predicting biogas production. Digested EFB were submitted for preliminary insect feeding trials. Subsequently, various domestic waste biomass was submitted for larvae feeding trials and analysed by NMR spectroscopy. Statistical models are being developed to determine the best mixture of these waste streams to produce the most nutritious substrate for larvae, providing an energy-neutral starting point for optimum insect rearing.

P-399 Unravelling the Flavour of White Asparagus using Metabolomics

PRESENTING AUTHOR: ***Eirini Pegiou, MSc., Netherlands***

CO-AUTHORS: ***Roland Mumm, Robert Hall***

Metabolomics has wide application in plant and food analysis. This includes variety differentiation, identification and monitoring of biomarkers for food quality and assessment of improved food products. Food processing often negatively impacts food quality attributes, such as flavour. Asparagus (Asparagus officinalis) is valued for its nutrients and characteristic flavour. Currently more than 30% of the harvested product is discarded as waste due to the harvesting process and strict market requirements.. However, this (waste) material could be useful for the production of asparagus powder for use as an ingredient in soups and sauces. With the current drying techniques the characteristic asparagus flavour is not well retained, provoking the need for additional artificial supplements. In this project we aim to evaluate the flavour profile of processed white asparagus materials, in comparison with fresh white asparagus. By analyzing products from different drying processes, we aim to define the optimal conditions which maintain maximum flavour. We use targeted and untargeted metabolomics approaches to evaluate asparagus aroma using GC-MS. Results reveal the complexity of the biochemical composition of fresh and cooked asparagus and how this is modified during processing. Interpreting the effect of different drying strategies on the asparagus aroma is helping us to define an optimal drying process, while contemplating food naturalness. The outcome of this project will enable current crop waste materials to be converted into high value and high quality dried food components.

P-400 The Livestock Metabolome Database: Enabling Livestock Metabolomics Research

PRESENTING AUTHOR: ***Seyed Ali Goldansaz, University of Alberta, Canada***

CO-AUTHORS: ***Susan Markus, Mark Berjanskii, Rupasri Mandal, Yan Meng, Hamed Pirimoghadam, Debjani Bhattacharyya Chowdhury, Ying Dong, Jiamin Zheng, Paul Luimes, Zhiquan Wang, Graham Plastow, David Wishart***

The application of metabolomics to livestock research lags far behind other applications of metabolomics. To further facilitate livestock metabolomics research, we have: 1) created a freely accessible online database called the Livestock Metabolome Database (LMDB; www.lmdb.ca) that contains comprehensive metabolomic data for 5 common livestock species (cattle, sheep, goats, horses, and pigs) and 2) performed a detailed experimental characterization of the sheep metabolome. The LMDB is expected to serve as a general hub to support metabolomics studies in livestock research. This database currently holds 1070 metabolites extracted from existing literature and our experimental data collected from studies of cow and sheep metabolomes. Our sheep metabolomic work focuses on profiling the blood metabolome of healthy sheep for marker-assisted prediction of economically important production traits including feed efficiency, carcass merit, pregnancy and litter size. Initial analyses for feed efficiency (n=250) using NMR, ICP-MS, and DI/LC-MS/MS have identified and quantified 191 serum/blood metabolites. A panel of 5 candidate biomarkers (AUC>0.7) distinguishes between lambs with high and low feed efficiency. In a second study, 158 serum metabolites were identified and quantified from pregnant sheep (n=500) with 3 candidate biomarkers (AUC=0.95) that differentiate pregnant from non-pregnant ewes, 35 days into gestation. We have also identified a different pattern in the serum metabolome between pregnant ewes carrying a single fetus versus those carrying multiples. These results are currently being validated using additional serum samples (n=900). These projects will expand the LMDB and help develop a "pen-side" metabolomic test to facilitate farm management practices and animal selection.

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PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-401 Change of volatile metabolites in strawberries (Fragaria ananassa) spoiled by fungi during decay

PRESENTING AUTHOR: ***SU-YEON NA, EWHA WOMANS UNIVERSITY, South Korea***

Strawberry (Fragaria ananassa) is one of the major fruits cultivated all over the world. However, it is highly susceptible to spoilage during shipping and storage, mainly due to food-borne pathogens. In this study, Cladosporium cladosporioides, which is known as a major pathogen of strawberries, was isolated from strawberries infected and inoculated onto disease-free strawberry fruits. After the inoculation of fungi, the decay of strawberries was measured every 12 hours for 3 1⁄2 days. Then, volatile metabolites produced by fungi grown on strawberry were analyzed and compared during decay periods. Volatile metabolites of the spoiled strawberries were extracted by solid phase micro-extraction (SPME) and analyzed by gas chromatography-mass spectrometry (GC-MS) during decay. Among them, 3-ethylbenzaldehyde, xylene, propan-2-yl butanoate, hex-2-enyl acetate, ethyl octanoate and propan-2-yl acetate were significantly reduced according to the decay periods. On the other hand, 2-methylbutan-1-ol, 3-methylbutan-1-ol, methyl 2-methylbutanoate, methyl (Z)-N-hydroxybenzenecarboximidate and caryophyllene gradually increased over decay periods. In particular, styrene, which is a well-known fungal volatile compound produced from phenylalanine, was produced at relatively high level and significantly increased from 48 hours to 60 hours. Also, data sets were processed by partial least squares-discrimination analysis (PLS-DA) to evaluate the differences in volatile metabolites according to the decay time. The application of PLS-DA revealed that the strawberry samples corrupted by C.cladosporioides could be distinguished according to decay time. Also, major volatile metabolites related to decay were determined. The significant volatile metabolites related to latter state of decay were benzaldehyde, styrene and 2-methylbutyl acetate.

P-403 Non-targeted mass spectrometry-based metabolomics approach for authentication of organic leaf vegetables

PRESENTING AUTHOR: ***Ka Yi MAN, The Hong Kong Polytechnic University, Hong Kong***

Vegetables are an important food source providing essential nutrients, fiber and bioactive phytochemicals. The global trend for consumption of organic vegetable, such as tomatoes, potatoes and leaf vegetables, was increasing rapidly over the last few decades because of the raising demand for healthier and safer diet as well as the raising concerns on environmental sustainability. Despite its profitable prospect and high demand in market, a reliable method to verify whether the plants are cultivated using organic practices is absence. By using non-targeted metabolomics approach, it is possible to acquire comprehensive information on the composition of the metabolite profiles that can be used to identify farming methods. In this research, four types of common leaf vegetables in Hong Kong (n=124), including Amaranthus tricolor L. (red amaranth), Brassica rapa var. parachinensis (choy sum), Ipomoea aquatica Forsk. (water spinach) and Lactuca sativa L. (lettuce), were collected from Hong Kong accredited farms and certificated organic farms. Non-targeted MS-based metabolomics analysis of aqueous and organic layers from liquid-liquid extraction reveals the difference in small molecules profile and lipid profile of leaf vegetables. The result from this study demonstrates that the species and farming methods can be discriminated by a set of primary metabolites and plant lipids.

P-404 Water availability in the soil and its effects on tropane alkaloid metabolism in Datura stramonium

PRESENTING AUTHOR: ***Fredd Vergara, German Centre for Integrative Biodiversity Research, iDiv, Germany***
CO-AUTHORS: ***Abigail Moreno-Pedraza, Gabriel Jennifer***

Datura stramonium is an annual plant that produces tropane alkaloids. Atropine and scopolamine are two of the most studied alkaloids in D. stramonium. Atropine and scopolamine are non-selective muscarinic acetylcholinesterase inhibitors. Atropine and scopolamine affect the central nervous system in animals and act as chemical defenses against herbivores. Molecules of atropine and scopolamine contain nitrogen. There is no evidence that D. stramonium stablishes symbiosis with nitrogen (N2) fixing bacteria. Thus, D. stramonium obtains nitrogen (NO3-) from the soil. The process of nitrogen assimilation requires the dissolving of NO3- in water. It is foreseeable that the availability of water in the soil influences the assimilability of NO3- by the roots of D. stramonium. In turn, the assimilability of NO3- can affect tropane alkaloids biosynthesis. To test this hypothesis we set up an experiment with different irrigations. To estimate the assimilability of NO3- we used tensiometers to quantify the soil water pressure. We analyzed different organs of D. stramonium grown under different irrigations using a non-targeted metabolomics approach (LC-qToF). We also determined the percentages of elemental carbon and nitrogen in different organs. Finally, we performed an absolute quantitation of atropine and scopolamine (LC-QqQ). We identified irrigations correlated with maximum production of tropane alkaloids. These findings are relevant in understanding herbivory patterns in nature.

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P-405 Effects of the climate conditions around the harvest days on the metabolites in the harvested edamame

PRESENTING AUTHOR: ***Akira Oikawa, Yamagata University, Japan***

CO-AUTHORS: ***Akira Oikawa, Yamato Horibe, Kei Morita, Katsutaka Takeuchi, Yasutaka Shimizu, Ryosuke Sasaki, Kazuki Saito, Hideki Murayama***

Quality of agricultural products is affected by long-term climate changes such as drought and high temperature. Local and short-term climate conditions also have effects on the ingredient in agricultural products. In this study, we focus on the relation between the metabolite changes in edamame (green soybean) and the climate conditions around the harvest days. Edamame was harvested every day of about 10 days for 6 years at the same field. Ionic metabolites and neutral sugars in the harvested edamame were comprehensively analyzed by using capillary electrophoresis mass spectrometer (CE-MS) and liquid chromatography mass spectrometer (LC-MS), respectively. The climate conditions around harvest days were also recorded on the every day. The results of the correlation analysis between the data of the edamame metabolome and the climate conditions revealed that the amounts of some metabolites in edamame were changed depending on the temperature and the day length around the harvest days. Especially, the amounts of lysine in the harvested edamame and the integrated temperature for 5 days before harvest were significantly correlated. Moreover, the amount of sucrose showed weak correlation with the length of day light. Furthermore, although it showed no significant differences, the temperature at the harvest day was inclined to be positively or negatively correlated to the amounts of several metabolites. These results showed the clear relations between the climate around the harvest day and the quality of the harvested edamame, which might lead to add values to agricultural products according to a new viewpoint.

P-406 A Fast LC-QqQ-MS/MS Method for the Quantification of Phytohormones in Stems of Forest Tree Species

PRESENTING AUTHOR: ***Ana Margarida Rodrigues, ITQB NOVA, Portugal***

CO-AUTHORS: ***Ana Margarida Rodrigues, Swen Langer, Ed Bergström, David Harvey, Tony Larson, Jane Thomas-Oates, Carla António***

Phytohormones are key low-abundant metabolites with a critical signaling role to internal and external cues, namely in mediating plant growth and development processes, adaptation to adverse environmental conditions or defence responses. Phytohormones belong to different classes, according to their chemical structure, and are present at very low concentrations in plants, which makes them challenging metabolites to quantify accurately. Thus, continuous improvements of sensitive analytical techniques able to quantify several phytohormones in a single run, and in different plant tissues, are needed. Moreover, most published analytical methods for quantitative analysis of phytohormones were validated for Arabidopsis or crop species, and very few were optimised for complex matrices as forest tree species. The aim of this study was to validate a simple, fast and sensitive analytical method for the quantitative analysis of 14 phytohormones, belonging to six major classes, in the complex matrix of Pinus pinaster Ait. stem tissues, using the triple quadrupole mass spectrometer LC-MS system (LC-QqQ-MS/MS) to take advantage of the high-resolution selected reaction monitoring (SRM) for higher sensitivity and selectivity. This method is fast, reliable, and sensitive, and allows the quantification of 14 phytohormones in a single run (6.6 min). Details on the key analytical validation steps will be presented, namely (i) establishment of calibration curves and assay linearity, (ii) assessment of matrix effects on metabolite quantification, (iii) assessment of the limit of detection and limit of quantification, (iv) assessment of the analytical recoveries during metabolite extraction, and finally (v) determination of the precision of the method.

P-407 Comparative metabolic profiling of soybean seeds from commercial cultivar (Glycine max) and wild soybean (Glycine soja)

PRESENTING AUTHOR: ***Sung-Dug Oh, Rural Development Administration, National Institute of Agricultural Sciences, South Korea***

CO-AUTHORS: ***Ancheol Chang, Sang Jae Suh, Soo-Yun Park***

Metabolomics is a useful tool for determining phenotypic variation in plants. This study aimed to identify the primary metabolites and bioactive secondary metabolites from seeds of wild soybean (Glycine soja) and Korean commercial cultivar (Glycine max, cv. Gwangan) and to explore metabolic differences between their genotypes. We profiled abundant hydrophilic primary metabolite and lipophilic secondary metabolites using gas chromatography-time-of-flight mass spectrometry (GC-TOFMS). Data obtained were subjected to multivariate statistical analyses, principal component analysis, orthogonal partial least squares discriminant analysis, and hierachical clustering analysis to determine phenotypic variation and relationships between metabolite contents. The identification and profiling of metabolites using GC-TOFMS analysis allows clear discrimination between soybean genotypes. This study determined comprehensive metabolic differences between soybean seeds with different genotypes and provides useful information for genetic manipulation of soybean to influence primary and secondary metabolism.

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PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-408 A GC-MS platform for assessing the diversity of fruit and leaf volatiles

PRESENTING AUTHOR: ***JOSÉ L. RAMBLA, Jaume I University. Department of of Agricultural and Environmental Sciences, Spain***
CO-AUTHORS: ***Aurelio Gómez-Cadenas, Antonio Granell***

Volatile compounds are important molecules in plants which are involved in several independent processes. Fruit volatiles have a role in the attraction of seed dispersers, and are key compounds in the human perception of fruit flavour. Vegetative tissues also modify their pattern of volatile emission in response to external factors such as the presence of insects and microorganisms, using them as a language for communication with other plant organs and with another plants, and also to communicate with other living organisms such as insects. Here we present some results obtained from our platform for the determination of plant volatiles based on capture by means of Headspace Solid Phase Microextraction (HS-SPME) and separation and detection by means of Gas Chromatography coupled to Mass Spectrometry (GC-MS). This platform was used for the characterization of volatile compounds in different fleshy fruit species, many of them with an impact in fruit flavour, and enabled the identification of genome regions and several genes involved in their biosynthesis. Additionally, a non-targeted approach also allowed the identification of volatile compounds involved in the response of vegetative tissues against both microbial and pest attacks, shedding some light in the mechanisms involved in plant defence.

P-409 Storability and variety-specific metabolite profiles of European onion landraces

PRESENTING AUTHOR: ***Christoph Weinert, Max Rubner-Institut, Germany***
CO-AUTHORS: ***C.H. Weinert, M. Häußler, M.L. Romo Pérez, B. Egert, M. A. Frechen, B. Trierweiler, C. Zörb, S.E. Kulling***

Onions are among the most popular and economically most important vegetables worldwide and contain a range of health-beneficial compounds. Under appropriate conditions, onions can be stored for up to nine months. Modern conventional farming heavily relies on the use of hybrid varieties which may lead to genetic erosion and a loss of biodiversity. The use of open-pollinated landraces and their further development by breeding may be a viable alternative, especially for organic farming. In this trial, we examined the metabolite profiles of nine landraces and a commercial control variety (Sturon) in the fresh state and after cold storage for up to five months at 2-3°C and <60% relative air humidity. Quantitative basic analyses as well as an untargeted GC×GC–MS analysis were performed. More than 200 onion metabolites were relatively quantified. In the fresh state, nine varieties could be separated into three groups, mainly according to differences in the sugar and amino acid profiles. The variety “Jaune des Cévennes” was characterized by remarkably low fructan levels and a higher content of monosaccharides. While this variety suffered from increasing Botrytis and Aspergillus infestations already after two month of cold storage, all other varieties demonstrated minimal water loss and no visual appearance of degradation even after five months. Storage lead, among others, to increased levels of glutamine, glutamate, asparagine, phenylalanine, serine, several sulfur-containing amino acids, fructose, sucrose, xylose, trehalose, raffinose, myo-inositol, phosphate, malate and a substantial decrease in, e.g., citrate, nystose and other di-, tri- and tetrasaccharides.

P-410 Increased throughput and coverage for the annotation of Saponins using a structure-based MSn approach on a Tribrid Orbitrap mass spectrometer

PRESENTING AUTHOR: ***Reiko Kiyonami, Thermo Fisher Scientific, United States***
CO-AUTHORS: ***Caroline Ding, Seema Sharma, Andreas Huhmer***

Saponins are major components of Chinese medicines and exert various pharmacological effects, such as cardiovascular protective activity and anticancer activity. Plus, they could reduce the side-effects of radiotherapy and chemotherapy. The comprehensive annotation of Saponins from various Chinese medicines remains challenging because of the limited availability of authentic standards and the structural diversity of this class of compounds. Taking advantages of high resolution MS and MSn capability offered by the tribrid Oribitrap mass spectrometer, we developed a product ion-dependent MSn data acquisition method in which MS2 data is constantly collected and further followed by higher order FTMSn if sugar neutral lose are detected from the MS2 data. The collected MSn tree data were used to identify the Saponin class compounds which contain the triterpenoid or spirostane aglycones. Chemspider and custom databases are further used for final saponin structure annotation. As the proof of concept, 80% methanol extracts from Sanqi, a Chinese medicine was analyzed. A Thermo Scientific™ Orbitrap ID-X™ Tribrid™ mass spectrometer was used for collecting HRAM MS and MSn (up to MS4) data. The MSn data were searched against msCloud spectral library using Thermo Scientific™ Mass Frontier ™ 8.0 software for identifying the saponin class compounds. The novel structure ranking tools included in the Thermo Scientific™ Compound Discoverer™ 3.0 software was used for final structure annotation of identified saponin class compounds. More than 60 sapanins were annotated from the Sanqi extract. The MSn data were critical for the identification of triterpenoid or spirostane aglycones.

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PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-411 NMR and Mass based metabolomics applied to the quality control of processed ginseng

PRESENTING AUTHOR: ***Dae Young Lee, Department of Herbal Crop Research, National Institute of Horticultural and Herbal Science, RDA, South Korea***

CO-AUTHORS: ***Dahye Yoon, Bo-Ram Choi, Jae-Suk Ban, Ryong Gong, Young-Seob Lee, Geum-Soog Kim***

The root of Panax ginseng C.A. Meyer has been widely used as ingredients for traditional medicines and functional foods. Furthermore, not only raw ginseng but also several processed ginseng products have been used in the food industry and herbal markets. According to the distinct processing methods, there are four processed P. ginseng products including white ginseng (WG), tae-geuk ginseng (TG), red ginseng (RG), and black ginseng (BG). In this study, HR-MAS NMR and UPLC-QTOF/MS-based metabolomics approaches were applied to assess the metabolic compositions of four processed ginseng. In the results of HR-MAS NMR, primary metabolites were identified such as sugars and amino acids. In the multivariate analyses of NMR spectra, PCA score plot showed that TG and RG were clustered each other, and discriminated from WG and BG. The main metabolites causing these cluster were sucrose and maltose. Maltose and sucrose were not detected in the WG and BG, respectively. In PCA score plot, four groups of processed ginseng were well differentiated. OPLS-DA was also performed to discriminate two selected samples. Several ginsenosides were found as the key components to differentiate four ginseng products. UPLC-QTOF/MS with an in-house library was used to profile ginsenosides from these four ginseng products. The numbers of identified ginsenosides from the four products were as follows: WG (n=26), TG (n=28), RG (n=34), and BG (n=36).

P-412 The effect of storage on the metabolite profile and antibactirial activity of Plectranthus madagascariensis

PRESENTING AUTHOR: ***Mhlonipheni Msomi, University of South Africa, South Africa***

CO-AUTHORS: ***Gerhard Prinsloo***

Plectranthus madagascariensis is a garden plant native to South Africa that is used to treat minor ailments. The study was conducted to investigate metabolite changes of P. madagascariensis using NMR based metabolomics and how it affects antibacterial activity against Staphylococcus aureus. Different storage conditions and the effect of storage conditions on the antibacterial activity of the plant were investigated. In the NMR-based metabolomics analysis, the supervised OPLS-DA separated samples that were stored in -80 C from those that were stored in the fridge and room temperature. Longer storage time of the leaf samples did not make a difference as the samples were scattered with no clustering observed. Drying conditions had no effect on metabolite profile in samples that were stored in different drying conditions. The resazurin-based assay demonstrated samples stored for one month was not active as most of them had a MIC of 1 mg/ml. The antibacterial activity of extracts tested after three months increased. Extracts that were stored in -80 C immediately after drying were unable to inhibit bacterial growth with the MIC values of 1 mg/ml. The best MIC value was 0.015 mg/ml for plants that were grown in the shade, dried in the sun and stored at room temperature. The extracts stored at room temperature showed different MIC values compared to extracts in the fridge, with generally better activity in samples stored at room temperature.

P-413 Terpenoid Diglycosides in the Salicaceae

PRESENTING AUTHOR: ***Alice Bellisai, Rothamsted Research, United Kingdom***

CO-AUTHORS: ***Gianluca Ruvo, Charlotte Lomax, Michael H. Beale, Jane L. Ward***

Willow is well known for the presence of phenolic glycosides, including the famous compound Salicin from which the well-known drug Aspirin was later developed. Terpenes, abundant in many plants are however less well known in members of the Salicaceae such as willow and poplar. Rothamsted Research maintains the National Willow Collection (NWC) as a short-rotation coppice plantation at Rothamsted Research. Comprising 1500 accessions, this germplasm resource is a rich source of novel chemistry which is being systematically screened by metabolomics. A family of novel terpene alcohol diglycosides have been identified which show a variety of carbohydrate components in their diglycoside moiety. Compounds have been isolated from Salix genotypes by HPLC and their structures were elucidated by LC-HRMS-MS and 1D and 2D NMR. We will demonstrate the diversity in both terpenoid and glycoside components, including data describing both pyranose and furanose forms. Finally, we will illustrate how particular molecules are species specific.

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P-414 Response of bush tea's (Athrixia phylicoides DC.) biochemical constituents to Kelpak® application using the LC-MS/MS triple quadrupole and 1H NMR techniques

PRESENTING AUTHOR: ***Keletso Mohale, University of South Africa, South Africa***

CO-AUTHORS: ***Kafua Lodama, Mudau Fathuwani Nixwell***

Biostimulants from seaweed such as Kelpak® (Ecklonia maxima O.) extracts have gained significance in agriculture and improved yields and quality of various crops. However, the effect of Kelpak® (E. maxima) on bush tea's (Athrixia phylicoides DC.) phytoconstituents has not been explored. The objective of this study was therefore to assess the influence of Kelpak® biostimulant on bush tea's chemical profile using LC-MS/MS triple quadrupole mass spectrometer and 1H nuclear magnetic resonance (NMR) spectroscopy. The treatments consisted of 0.2%, 0.1%, 0.05% Kelpak® and control (0.0%) arranged in a completely randomized design (CRD). Kelpak® application stimulated bush tea plant height and increased number of lateral branches produced. The study also revealed that bush tea's primary metabolites including amino acids, organic acids and vitamins were enhanced by application of 0.2% Kelpak®. The partial least squares–discriminant analysis (PLS-DA) demonstrated four distinct groups of bush tea samples treated with different levels of Kelpak®. Based on the 1D NMR spectrum prediction putative annotation of metabolites resulted in detection of 32 metabolites with varying intensities. The application of 0.2% Kelpak® demonstrated high intensities of the detected compounds and could thus be recommended for improved bush tea plant growth and biochemical constituents.

P-416 The Metabolic Characteristics Analysis of Tobacco Leaves from Different Flavor-Style Locations

PRESENTING AUTHOR: ***Pingping Liu, Zhengzhou Tobacco Research Institute of CNTC, China***

CO-AUTHORS: ***Liu Pingping, Chen Qiansi, Zhang Hui, Zheng Qingxia, Xu Guoyun, Zhai Niu, Wang Chen, Jin Lifeng, Cao Peijian, Zhou Huina***

Metabolomics can be used to characterize the metabolites after various internal and external environmental disturbances (gene changes or environment changes)[1-2]. Metabolites are the material basis of formation of tobacco flavor. The flavor of tobacco directly affected by the changes of metabolites concentration. Before the previous research, the flavor of tobacco has a high correlation with the location and the climate environment. In this study, the mature tobacco leaves (middle leaf of fresh tobacco at maturity stage) from 20 sampling sites were collected (including three traditional flavor types) on a national scale, and the metabolic characteristics of these samples were analyzed. The characteristic metabolites which were closely related to flavor style were investigated. The result revealed that, 21 metabolites were related to heavy-flavor type, 16 metabolites were related to fen-flavor type and 10 metabolites were related to neutral-flavor type. The metabolic pathways enrichment results indicated that, the lipid metabolism level of heavy-flavor tobacco leaves was higher, especially the glycolipid metabolism. The triglyceride metabolism level of fen-flavor tobacco was higher and the sugar metabolism level was low. The ascorbic acid salt synthesis and metabolism of neutral-flavor type tobacco leaves was also strong.

P-417 Metabolomic analysis of headspace volatiles of Plectranthus neochilus Schltr. (Lamiaceae)

PRESENTING AUTHOR: ***Alexandra Sawaya, FCF-UNICAMP, Brazil***

CO-AUTHORS: ***Maria Isabel Galbiatti, Guilherme Perez Pinheiro, Elisa Ribeiro Antunes, Vinícius Veri Hernandes, Marcos Nogueira Eberlin***

Plectranthus neochilus Schltr. (Lamiaceae) is popularly known as boldo in Brazil and used for digestive problems, similar to the Chilean plant, Peumus boldus. The essential oil was proven to be schistosomicidal, antimicrobial and reduced whitefly colonization in tomato crops. Despite these promising activities, there were no studies in the literature on the variability of its essential oil throughout the year. Samples were collected from four individuals grown in the field (monthly, morning and afternoon) and inside a greenhouse. All the collected leaves were immediately frozen. Prior to analysis the samples were crushed in liquid nitrogen, a 500 mg aliquot of each was sampled by SPME fiber (PDMS / DVB) and inserted in GC-MS (Agilent) using an automatic injector (splitless), temperature gradient 60 -246 ° C at a rate of 3 ° C / min. QC samples were analyzed every 16 samples. The chromatograms were aligned and the features extracted by XCMS-Online, chemometrics by Online MetaboAnalyst and GraphPad Prism 6.01 software for ANOVA. One compound, 1-octen-3-ol was a marker for the afternoon samples in the field and the greenhouse samples for all individuals. This compound is present in edible mushrooms, but in plants only one study related it activation of defense genes. Possibly high solar incidence in the afternoon and high temperatures in the greenhouse caused environmental stress for P. neochilus plants. There was, however, no significant monthly variation of the volatiles, indicating that it is possible to harvest leaves throughout the year.

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***AWARD WINNERS**

PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-418 Metabolic responses to potassium availability and waterlogging reshape respiration and carbon use efficiency in oil palm

PRESENTING AUTHOR: ***Jing Cui, Australian National University, Australia***

CO-AUTHORS: ***Marlène Davanture, Michel Zivy, Cathleen Mirande-Ney , Emmanuelle Lamade, Guillaume Tcherkez***

Oil palm is a strong potassium (K)-demanding species cultivated in regions where soil K availability is generally low and waterlogging caused by tropical heavy rains can limit further nutrient absorption. However, the metabolic effects of K and waterlogging have never been assessed precisely. This is very surprising because most tropical or wet areas where rice, sunflower, oil palm and other important crops are cultivated combine these two environmental constraints. The aim of this study was to understand the overall impact of waterlogging and limited K availability on metabolism, and to understand how such advert environmental conditions reshape the carbon balance in oil palm. Here, we examined the metabolic response of oil palm saplings in the greenhouse under controlled conditions (nutrient composition with low or high K availability, with or without waterlogging) using gas exchange, metabolomics and proteomics analyses. Our results show that both low K and waterlogging have a detrimental effect on photosynthesis but clearly stimulate leaf respiration, thereby impacting the carbon use efficiency. Omics analyses show differential accumulation of typical metabolic intermediates and enzymes not only of the Krebs cycle but also of alternative catabolic pathways. In addition, we found a strong relationship between metabolic composition and the rate of leaf dark respiration. Overall, advert environmental conditions have an enormous impact on respiration in oil palm. Leaf metabolome and proteome appear to be good predictors not only of K availability but also of CO2 efflux, and this opens avenues for cultivation biomonitoring using functional genomics technologies.

P-419 Assessment of the arsenic exposure in rice (Oryza sativa japonica): Combining untargeted metabolomics and lipidomics

PRESENTING AUTHOR: ***Miriam Pérez Cova, IDAEA-CSIC, and Universitat de Barcelona, Spain***

CO-AUTHORS: ***Miriam Pérez-Cova, Romà Tauler, Joaquim Jaumot***

Arsenic polluted soils have a direct effect on edible plants-base foods such as rice (Oryza sativa japonica). Metabolomics and lipidomics strategies aid to achieve a better understanding of how arsenic is assimilated and translocated, and the main metabolic pathways affected by this pollutant. Two As exposures were applied during the first three weeks of rice growth in an environmental chamber: by irrigation (1 or 1000 µM) and settled in soil before planting (5 or 50 ppm). Aerial parts and roots were collected, extracted (using two extraction protocols, for metabolites and lipids) and analyzed separately. Metabolomics LC-MS analysis was conducted using a TSK Gel Amide-80 HILIC column and a Thermo OrbitrapQExactive mass spectrometer operating in both, Full MS and All Ion Fragmentation mode. Lipidomics LC-MS analysis employed a Kinetex C8 column and a Waters LCT PremierToF. Ionization source for metabolomic and lipidomic analysis was Electrospray in positive and negative mode. Workflow for metabolomics (W4m) platform was used for preprocessing, and multivariate tools (PCA, PLS-DA, ASCA) from Metaboanalyst were employed in the subsequent data analysis. Results obtained after chemometric analysis show that only the highest As concentration levels (both in irrigation water or settled in soil) allowed the differentiation when compared with control samples (without arsenic treatment). No clear differentiation was found between the low concentration samples from both treatments and control samples. Finally, metabolomics and lipidomics results were integrated to have a more comprehensive overview of As exposure effects in rice, by combining the new hypotheses coming from the detected compounds.

P-420 A novel method for identification and quantification of sulfated flavonoids in plants by neutral loss scan mass spectrometry

PRESENTING AUTHOR: ***Sabine Metzger, University of Cologne, Germany***

CO-AUTHORS: ***Niklas Kleinenkuhnen, Felix Büchel, Silke C. Gerlich, Stanislav Kopriva***

Sulfur is present in plants in a large range of essential primary metabolites, as well as in numerous natural products. Many of these secondary metabolites contain sulfur in the oxidized form of organic sulfate. However, except of glucosinolates, very little is known about other classes of such sulfated metabolites, mainly because of lack of specific and quantitative analytical methods. We developed an LC-MS method to analyse sulfated flavonoids, a group of sulfated secondary metabolites prominent, e.g., in plants of the genus Flaveria. The method uses a linear gradient of methanol/formic acid in water on a Restek Raptor C18 Core-Shell column for separation of the compounds. The sulfated flavonoids are detected by mass spectrometry (MS) in a negative mode, using a neutral loss of 80 Da after a collision induced dissociation. With this method we were also able to quantify the sulfated flavonoids. We could detect all (mono)sulfated flavonoids described before in Flaveria plus a number of new ones, such as isorhamnetin-sulfate-glycoside. In addition, we showed that sulfated flavonoids represent a substantial sulfur pool in Flaveria, larger than the thiols glutathione and cysteine. The individual species possess different sulfated flavonoids, but there is no correlation between the qualitative pattern and type of photosynthesis. Similar to other sulfur-containing secondary compounds, the concentration of sulfated flavonoids in leaves is reduced by sulfur starvation. The new LC-MS method will enable qualitative and quantitative detection of these secondary metabolites in plants as a pre-requisite to addressing their functions.

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***AWARD WINNERS**

PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-421 Construction and new methodology research on untargeted metabolomics database of tabacoo

PRESENTING AUTHOR: ***Zheng Qingxia, Zhengzhou Tobacco Research Institute of CNTC, China***

CO-AUTHORS: ***Peng Lu, Yongjie Yu, Lifeng Jin, Pingping Liu, Qiansi Chen, Niu Zhai, Guoyun Xu, Hui Zhang, Jingjing Jin, Peijian Cao, Huina Zhou***

Metabolomics, a powerful tool to investigate the relationship between genotype and phynotype, attracts an increasing amount of attention. Ultra-high performance liquid chromatography combined with high-resolution mass spectrometry, such as UPLC-QTOF, has become a dominant technique in metabolic studies, particularly for untargeted metabolomics. An obvious advantage of UPLC-QTOF-based untargeted metabolomics is that thousands of components can be simultaneously analyzed, possessing two great challenges for users to data analysis and identify metabolites. Toward these challenges, we constructed an automatic UPLC-HRMS data analysis software, named AntDAS and a freely accessible metabolite database, named TMMLib. AntDAS can be perform data analysis to effective and efficiently extract features of metabolites in samples, whose output involving high-accuracy m/z values, retention time can be directly imported into the TMMLib for compound identification. TMMLib includes an annotated list of known metabolite structural information and their MS-MS spectra from tobacco. TMMLib can realize single and batch searches, as well as MS-MS searches. AntDAS and TMMLib will be regularly updated and to greatly benefit future metabolomics studies in plant .TMMLib is freely available at http://tmmlib.tobaccodb.org and the AntDAS code is available from http://software.tobaccodb.org/software/antdas.

P-422 Leaf metabolite profiling reveals biochemical diversities within wheat genetic resources

PRESENTING AUTHOR: ***Annick Moing, INRA Bordeaux, France***

CO-AUTHORS: ***Pierre Petriacq, Amelie Flandin, Sylvain Prigent, Cedric Cassan, Stephane Bernillon, Dominique Rolin, Renaud Rincent, Christophe Salon, Christian Jeudy, Jacques Le Gouis, Yves Gibon, A. Moing***

BreedWheat (www.breedwheat.fr) and ArchiRac (www.fsov.org) are French national projects aiming at providing new tools and materials for wheat breeding, thereby increasing and facilitating the use of original genetic resources to improve allelic variability in the elite wheat (Triticum aestivum L.) gene pool. A large panel of elite winter-wheat varieties was selected to cover the diversity present in French material and phenotyped in multi-environment trials to identify tolerance traits and QTLs within the BreedWheat project. In addition, two young plants per genotype were cultivated in controlled conditions within specially designed containers (Jeudy et al. 2016, Plant Methods 12: 31) for high-throughput root-phenotyping for three weeks of vegetative growth within the ArchiRac project. Six reference genotypes were used to account for block variation in the experimental design. The largest fully-emerged leaf of each plant was harvested in the morning, rapidly frozen and lyophilized for biochemical studies. Robotized targeted analyses of major metabolites and liquid chromatography coupled to mass spectrometry (UHPLC-Orbitrap-MS) were used to assess the biological variation of primary and specialized metabolites as well as starch and total protein content. Multivariate and univariate statistical analyses were used to mine the profiles of all genotypes. Compositional distances between genotypes were calculated and visualized using clustering in order to compare metabolic and molecular similarities. Metabolomic data will be further combined to other phenotyping data to search for metabolic biomarkers that will link to plant performance. Acknowledgements: MetaboHUB (ANR-11-INBS-0010), PHENOME (ANR-11-INBS-0012), BreedWheat (ANR-10-BTBR-03) and ArchiRac (FSOV 2016K) projects for financing.

P-423 Metabolic flux may active through freeze drying process of tobacco leaves

PRESENTING AUTHOR: ***Huina Zhou, Zhengzhou Tabacco Research Institute of CNTC, China***

CO-AUTHORS: ***Qiansi Chen, Pingping Liu, Qingxia Zheng, Niu Zhai, Huina Zhou***

To fully investigate the effects of vaccum freeze drying on the metabolome of tobacco leaves, the fresh-frozen samples in liquid nitrogen were divided into 3 aliquots, one kept at frozen as fresh samples and the left two freeze-dried under controlled or uncontrolled shelve surface temperature as FD1 and FD2 samples, respectively. Three kinds of samples were balanced in equal dry weight, and analyzed by GC-MS and LC-Q-TOF with six replicates to collect their metabolome data. A total of 79 and 99 metabolites were identified by GC-MS and LC-Q-TOF, respectively. PCA score plots showed that freeze-dried smaples (FD1 and FD2) did different from fresh-frozen samples in metabolomics level, and LC-Q-TOF analysis also revealed a clear difference resulted by controlled shelve surface temperature. Furthermore, based on VIP list and T-test analyses, a tendency was found that different metabolites identified by GC-MS usually decreased upon freeze drying, while different metabolites identified by LC-Q-TOF were mostly increased. Considering the category of different metabolites, sugars, organic acids and amino acids usually down-regulated, while terpenoids and polyphenol/flavonoids usually up-regulated by freeze-drying, the different in metabolome after freeze-drying may result by the active metabolic flux from primary metabolites to secondary metabolites, besides the volatilization of some volatile compounds. And we also found that freeze-drying with controlled shelve surface temperature might slow down the metabolic flux, since the total levels of different metabolites in FD 1 were closer to Fresh samples other than FD2.

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***AWARD WINNERS**

PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-424 A GWAS study of Arabidopsis secondary metabolism

PRESENTING AUTHOR: ***Marlies Brouckaert, VIB, Belgium***

CO-AUTHORS: ***Rene Hofer, Geert Goeminne, Veronique Storme, Yvan Saeys, Kris Morreel, Wout Boerjan***

A combination of high-throughput metabolomics with genome-wide association studies (GWAS) can contribute to the identification of genes underlying metabolic diversity and their relevance to complex traits, such as metabolite levels, in different accessions of the same species. In this research project, the metabolic profiles of 14-day-old seedlings from 225 Arabidopsis thaliana accessions in 5 biological replicates were analyzed by untargeted liquid chromatography-mass spectrometry. Across all chromatograms, 4479 m/z features segregated as binary traits, i.e. being absent or present. Co-segregation analyses with SNPs, obtained from a previously published 250k SNP chip data set (Horton et al., 2012), is currently being performed. In total, 1311 associations (Fisher's exact test P value < 1x10-10) containing 1046 unique genes were found between the SNPs and absence/presence of 194 m/z features. Focusing on the structurally characterized m/z features, 3 glucosinolates were associated to the MAM2 locus, known to be involved in the biosynthesis of this class of metabolites. In addition, an association between three characterized flavonol-glycosides and the BGLU6 gene was found. Moreover, an association between isoleucin and malonyl-isoleucin and DAAR1 could be retrieved. Supported by these previously proven associations, this method will be applied to find new gene-metabolite links. Horton, M.W. et al. Genome-wide patterns of genetic variation in worldwide Arabidopsis thaliana accessions from the RegMap panel. Nature genetics 44, 212-6 (2012).

P-425 Comparative metabolite analysis between Lycoris radiata and Narcissus tazetta

PRESENTING AUTHOR: ***Chang Ha Park, Department of Crop Science, Chungnam National University, South Korea***

CO-AUTHORS: ***Sang Un Park***

Comparative metabolite analysis between Lycoris radiata and Narcissus tazetta. Chang Ha Park and Sang Un Park* - Department of Crop Science, Chungnam National University, 99 Daehak-ro, Yuseong-gu, Daejeon 34134, Korea. This study aimed to comprehensively examine the interface between primary and secondary metabolites in Lycoris radiata (L. radiata) and Narcissus tazetta (N. tazetta), using gas-chromatography coupled with time-of-flight mass spectrometry (GC-TOFMS) and high-performance liquid chromatography (HPLC). There was significant variation of chemical composition in the different parts of L. radiata and N. tazetta. In particular, The level of galantamine was highest in leaves of L. radiata. However, bulbs of N. tazetta contained the highest level of galantamine. Primary and secondary metabolites identified by GC-TOFMS and HPLC were subjected to partial least-squares discriminant, Pearson's correlation, and hierarchical clustering analyses, which indicated significant differences in the primary and secondary metabolisms of L. radiata and N. tazetta. This metabolome study comprehensively describes the relationship between primary and secondary metabolites in L. radiata and N. tazetta and provides the information useful for developing strategies to enhance the biosynthesis of galantamine in Amaryllidaceae plants. Additionally, This work highlights that HPLC and GC-TOFMS-based metabolite profiling is suitable techniques to evaluate morphological variation and determine metabolic differences in L. radiata and N. tazetta. *(Corresponding author) E-mail: supark@cnu.ac.kr, Tel: +82-42-821-6730. (Acknowledgement) This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Ministry of Science, ICT & Future Planning (2016M3A9A5919548).

P-426 Integrated metabolome and transcriptome analysis of plants identifies biosynthetic pathways for floral volatile organic compounds

PRESENTING AUTHOR: ***In-Cheol Jang, Temasek Life Sciences Laboratory, Singapore***

CO-AUTHORS: ***Savitha Dhandapani***

Plants emit a number of floral volatile organic compounds (VOCs) as secondary metabolites that have ecological functions for their defence against herbivores and pathogens as well as for the pollinator attraction. Plant VOCs are mainly composed of terpenoids, phenylpropanoids/benzenoids, and volatile fatty acid derivatives, which are derived from different biosynthetic routes in plants. The diversity of plant VOCs has stimulated broad systems biology approaches to identify the pathways/genes involved in their biosynthesis. We integrate metabolome and transcriptome analysis of aromatic flowers to unravel biosynthetic pathways for floral VOCs. Our metabolite-guided transcriptomics and molecular and biochemical characterization of genes have identified specific gene members encoding enzymes involved in the biosynthesis of diverse floral scents. Here, we will talk about our recent progress on genes/pathways identification for biosynthesis of floral scents.

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***AWARD WINNERS**

PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-427 A metabolomics profiling of a traditional Chinese medicine Radix Stemonae

PRESENTING AUTHOR: ***Chunping Tang, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, China***

CO-AUTHORS: ***Shuai-Zhen Zhou, Chang-Qiang Ke, Yang Ye***

Natural products have been a major resource of new drugs due to their highly diverse structures and biological activities. Searching for natural products with the potential to be lead compounds from traditional Chinese medicines (TCMs) have drawn more and more attention. A metabolomics investigation was carried out on a commonly used TCM Radix Stemonae, from which an array of alkaloids reflecting its therapeutic effects were discovered. Radix Stemonae, known as Baibu in Chinese, is a commonly used antitussive agent. The Chinese pharmacopeia documents three species, Stemona tuberosa, S. sessilifolia and S. japonica as its original plants, and spring and autumn as the collecting seasons. Our previous investigations have revealed that the Stemona alkaloids are the characteristic secondary metabolites responsible for the therapeutic effects. In this study, a metabolite profiling method based on the UPLC-QTof technique was established and applied for 218 samples of crude alkaloids prepared from three Stemona species collected in different localities and seasons. PCA and PLS-DA models were used to analyze the data. The results showed that the alkaloidal constituents vary greatly with species and localities while little with harvesting seasons. The PCA model can be successfully applied to identify the species of unknown Stemona samples. Chemical markers of each species were identified by using an PLS-DA model. The results further revealed that the characteristic alkaloid of S. sessilifolia collected in Shandong province is different from that collected in Anhui province. The findings provide scientific evidence to support traditional descriptions of Baibu in medicinal books.

P-428 An evaluation of different ripening conditions and postharvest treatments in mangosteen (Garcinia mangostana) using metabolomics approach

PRESENTING AUTHOR: ***Anjaritha Aulia Rizky Parijadi, Osaka University, Japan***

CO-AUTHORS: ***Sobir Ridwani, Fenny M. Dwivany, Sastia P. Putri, Eiichiro Fukusaki***

Mangosteen is a tropical fruit with a high market value but a relatively short shelf-life. Despite being one of the most important tropical fruits, evaluation of different ripening condition and evaluation the effect after postharvest treatment to prolong shelf-life using metabolomics approach in mangosteen have never been studied. The aims of this study were to evaluate the metabolic changes between different harvesting and ripening condition and to evaluate the effect of postharvest treatment in mangosteen. Mangosteen ripening stage were collected with several different conditions (“on-tree”, and “off-tree”). The metabolite changes were investigated for each ripening condition. Additionally, fruit was harvested in stage 2 and was treated with several different treatments (storage at low temperature (LT; 12.3 ± 1.4 °C) and stress inducer treatment (SI; methyl jasmonate and salicylic acid) in comparison with control treatment (normal temperature storage) and the metabolite changes were monitored over the course of 10 days after treatment. Our findings clearly indicate that there is a similar trend of metabolic changes (the accumulation of some aroma precursor metabolites and firmness-related metabolites in the flesh and peel part, respectively) between two ripening conditions although the progression of “off-tree” ripening process observed through color changes occurred faster compared to “on-tree” ripening. Additionally, the metabolome data and color changes observation showed that LT treatment could prolong shelf-life among all treatments in all fruit part. It is the first report of the utilization of metabolomics to support postharvest development strategies in mangosteen.

P-429 Effect of water restriction and rehydration on flavonoid profile in leaves of Eucalyptus urophylla

PRESENTING AUTHOR: ***Hana Karina Pereira da Silva, University of São Paulo, Brazil***

CO-AUTHORS: ***Thaís Regiani Cataldi, Carlos Alberto Labate***

Eucalyptus plantations have a great economic and environmental importance in Brazil. Considering the climate change scenario and the cumulative precipitation deviations observed in these plantation areas in recent years, it is essential to understand how plants respond to stressful conditions. Drought is one of the main factors limiting plant yield and survival. Plants under water deficit can increase their reactive oxygen species content culminating in cell death. Under such conditions, the biosynthesis of flavonoids is induced, suggesting their important role as an auxiliary antioxidant system. This system is very important in plants under severe or multiple stresses once it can help these organisms cope with the given situation. In this work, leaves of two commercial clones (AEC144 and IPB1) of Eucalyptus urophylla under water restriction and rehydration were evaluated in terms of physiological and biochemical parameters. In addition, flavonoids profiles were analyzed combining a flavonoid enrichment extraction method and LC-MS/MS approach. Water limitation provoked alterations in relative water content and in lipidic peroxidation. On the other hand, alterations in these two parameters were not significant when both rehydrated and control plants were compared. When plants under distinct water conditions and genotypes were compared (by volcano plot - MetaboAnalyst), we found differentially abundant metabolites (DAMs). The exact mass of these DAMs corresponding to the target flavonoids (quercetin, luteolin, kaempferol, apigenin and their derivatives). At this moment, we are working on the structural elucidation of these target metabolites by MS/MS.

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***AWARD WINNERS**

PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-430 **Large-scale screening of carotenoid and chlorophyll content in tomato cultivars by using the simple, rapid and quantitative method**

PRESENTING AUTHOR: ***Yusuke Aono, University of Tsukuba, Japan***

CO-AUTHORS: ***Yonathan Asikin, Ning Wang, Harry J Klee, Miyako Kusano***

Tomato is one of the highest-value crops worldwide. Modern tomato cultivars head to add commercially attractive factors, including flavors. Wild tomato species, e.g., Solanum lycopersicum ver. cerasiforme and S. pimpinellifolium, have a great potential to produce useful volatiles because of their large genomic diversities. Previous study revealed that presence of volatiles in tomato fruits can be improved for taste of sweetness and sourness. Furthermore, it contributes to add favorite flavors for tomato consumers. Of these, apocarotenoid volatiles, which are one of the carotenoid derivatives, are enzymatically cleaved by carotenoid cleavage oxygenases. Additionally, these volatiles have a large impact on the perception of flavor. Therefore, it is important to investigate novel factors in the biosynthetic pathway for production of apocarotenoid volatiles. However, it remains unclear what kinds of key enzymes and/or transcription factors contributing to produce apocarotenoid volatiles that have useful aroma. In this study, we focused on the extent of chemical diversities in tomato cultivars to possess great potential to produce useful volatiles derived from carotenoid pathway. To achieve it, we developed the high-throughput screening method to quantify important pigments, i.e., lycopene and total carotenoid by using microplate reader. We also quantified chlorophylls because it is expected that we could be predict tomato ripening stages. We assayed 362 tomato cultivars including S. lycopersicum, S. lycopersicum ver. cerasiforme, and S. pimpinellifolium by using our developed system. We will present what kinds of tomato cultivars contain specific content of total carotenoids, lycopene and chlorophylls in the assayed tomato fruits.

P-431 **Creating the DynLib Mass Spectrometry Database of the Maize Secondary Metabolome using the RDynLib package**

PRESENTING AUTHOR: ***Sandrien Desmet, VIB, Belgium***

CO-AUTHORS: ***Yvan Saeys, Rebecca Dauwe, Hoon Kim, Geert Goeminne, Ruben Vanholme, John Ralph, Kris Morreel, Wout Boerjan***

The importance of maize for the production of food and industrially relevant products, e.g. bio-ethanol, as well as the enormous genetic diversity, a vast collection of mutant stocks and the high degree of genomic collinearity with other cereal crops, has led to the construction of several gene/transcript and protein databases, such as gramene and MaizeGDB. However, Mass Spectrometry (MS) spectral databases of especially the secondary metabolome of maize are lagging behind. Here, we present the DynLib MS spectral database for maize that was generated using different tissues (stem, leaf, ear, tassel and cob). The database contains both Quadrupole-Time-of-Flight-based MS/MS and Fourier Transform-Ion Cyclotron Resonance-Ion Trap-based MSn spectra from both the known and unknown Liquid Chromatography (LC)-profiled compounds. The RDynLib package was used to associate MS/MS and MSn spectra belonging to the same compound. Implementing RDynLib into our structural elucidation pipeline led to the characterization of 428 compounds. Based on this set of characterized compounds, we putatively annotated the maize- and tissue-specific conversions that are responsible for the prevailing mass differences that were picked from the LC-MS data.

P-432 **Study on anti-depressive constituents and mechanism of Herbal medicine, Chai-hu Shu Gan San**

PRESENTING AUTHOR: ***Zhong-Mei Zou, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, China***

CO-AUTHORS: ***Zhong-Mei Zou, Meng Yu, Hong-Mei Jia***

It is universally acknowledged that the complexity of TCMs makes the identification of the chemical constituents related to the efficacy and definition of their mechanism of action challenging. Novel approaches are in great demand to provide deeper mechanistic insight into the clinical valuable effects. Metabolomics, as one of the 'omics' technologies of systems biology, is the comparative analysis of metabolites and their dynamic flux associated with the response of living systems to pathophysiological stimuli or genetic modification. By means of advanced analytical tools, including nuclear magnetic resonance (NMR), and mass spectrometry (MS), in conjunction with the multivariate data analysis (MVA), metabolomics approach has been extensively applied in many areas such as diagnosis and treatment of disease, drug toxicity, biomarker discovery, and exploration of pathogenesis. Chai-hu Shu Gan San Chaihu-Shu-Gan-San (CSGS), a traditional Chinese medicine (TCM) formula containing seven herbal medicines, has been used in treatment of gastritis, peptic ulcer, irritable bowel syndrome and depression clinically. This presentation will focus on investigation into its active constituents and mechanism of anti-depressive effect by using LC-MS/MS and HNMR metabolomics.

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***AWARD WINNERS**

PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-433 EcoMetEoR - An enlightning platform for eco-metabolomic research

PRESENTING AUTHOR: ***Henriette Uthe, Friedrich Schiller University Jena, Germany***

CO-AUTHORS: ***Alexander Weinhold, Fredd Vergara, Nicole M. van Dam***

EcoMetEoR - An enlightning platform for eco-metabolomic research. Eco-Metabolomics is a relatively new research discipline and describes the application of metabolomics techniques to ecology with the aim to characterize biochemical interactions of organisms across different spatial and temporal scales 1. Changes in metabolite concentrations can provide mechanistic evidence for biochemical processes that are relevant at ecological scales. These include physiological, phenotypical and morphological responses of plants and communities to environmental changes and interactions with other organisms, too. Although, eco-metabolomics is a powerful tool to combine extrinsic ecological research and intrinsic biochemical research, biodiversity researchers are often limited in applying metabolomics due to lack of facilities and expertise. In addition, ecological research, especially in the field comes along with challenging sample quality and data analysis. To overcome this lack the EcoMetEoR platform uniquely focuses on supporting and teaching ecologists and biodiversity researchers to implement metabolomics analyses in their research programs. This support covers the experimental design, the accsess to the analytical equipment, sample preparation and data analysis and teaching during workshops and individual research stays.

P-434 Metabolomics as a Discovery Tool for Bioprospecting and Detection of Defense Compounds During Fungal Infection of Spruce Wood

PRESENTING AUTHOR: ***Marie-Pierre Pavageau, Thermo Fisher Scientific, France***

CO-AUTHORS: ***Anas Kamleh, Marit Almvik, Nina Elisabeth Nagy, Hans Ragnar Norli, Ari Hietala, Sven-Roar Odenmarck, Monica Fongen, Claire Dauly***

Every fourth tree in the Scandinavian forest is infected with rot. Root and butt rot fungi attack trees through the root and successively grow into the trunk degrading the core wood. The infected log can no longer be used as construction materials, damaging its value. We have employed tree metabolome analyses to differentiate and identify defense metabolites in Norwegian Spruce for new fungicide discovery as well as discovery of novel pathways that could be targeted by pesticides. Wood cores from healthy and white rot infected (Heterobasidion parviporum) spruce trees were sampled at trunk heights 0, 1.6 and 3.2 meters and from different zones of the core. A total of 73 samples were extracted and analyzed using LC connected to Q-Exactive(TM) and Orbitrap-IDX(TM) Mass spectrometers. Data were processed using Compound Discoverer(TM) software which allows the pre-processing and statistical analysis of data within the same platform as well as including tools for compound identification such as database and library matching. Compound identification was carried out by matching MS2 spectra and retention time to authentic standards, when feasible, or were tentatively annotated based on molecular formula search against compound databases or MSMS matching against mzCloud spectral library. We also looked at compounds that displayed high abundance in the fungal infected core wood as opposed to the reaction zone and found a new compound in spruce with composition C12H8O7, tentatively identified as purpurogallin-4-carboxylic acid, which we believe is a product from the fungal detoxification of wood defense compounds.

P-435 Metabolomic studies on conservation in response of cereal secondary metabolism to fungal infection

PRESENTING AUTHOR: ***Natalia Witaszak, Institute of Plant Genetics Polish Academy Sciences, Poland***

CO-AUTHORS: ***Anna Piasecka***

Several Fusarium spp., are economically devastating pathogens of cereals causing Fusarium head blight (FHB) which leads to reduction in crop yield world-wide. In addition, production of mycotoxins in cereal grain can be harmful to animals and humans. Elaboration of strategies for engineering resistance in cereal crops has to be boost by detailed knowledge about plant response against FHB at the molecular level. Untargeted metabolomics based on LC-MS was applied for studying the conservation of plant immunity response on pathogen infection at metabolomic level among economically important crops barley and wheat as well as taxonomically related model plant Brachypodium distachyon. MZmine2, MetaboAnalyst and MarVis software were used for defining the immunity-related secondary metabolites. Response of metabolomics signals was highly species-specific. Only small percent of differentially accumulated metabolites (DAMs) were common for all studied species. However, conservation of cereals immune system manifested in the similarity of metabolic pathways responding to infection. The most significant changes were observed for compounds annotated to metabolic pathway of tryptophan, especially in Brachypodium. In addition, metabolism of alkaloid gramine and polyamines was re-programmed during infection in all studied plants. Phenylpropanoids, mainly ferulic and p-coumaric acids and its conjugates with polyamines were the most numerous DAMs common among species. LC-MS - based metabolomics proved to be excellent tools for studying the basics of plant immunity reactions to fungal pathogens in closely related species. This study was supported by the National Science Centre grant Sonata 2015/17/D/NZ9/03347

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***AWARD WINNERS**

PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-436 Effect of growing conditions on the tomato root exudate and its associated root microbiome

PRESENTING AUTHOR: ***Bora Kim, University of Amsterdam, Netherlands***

CO-AUTHORS: ***Bora Kim, Marco Bentlage, Benjamin Thiombiano, Harro Bouwmeester, Anouk Zancarini***

The host-microbiome interaction is important for host functions and health. While many endeavors were made to understand how the host shapes its microbiome in the human gut, the effect of plant metabolite exudation on the rhizosphere and root microbiome is still unclear due to the technically challenging root exudate collection and analysis. Indeed, plant root exudates are mostly studied under artificial conditions, such as in hydroponics, to avoid unwanted contaminants from natural soils. However, several questions are often arising with regard to the similarity of root exudate profiles under artificial and natural conditions. To answer these questions, we compared plant root exudate composition under aeroponics and two natural soil conditions using an untargeted metabolomics approach. The results showed that up to 29% of compounds in the root exudate were quantitatively different depending on the growing condition indicating that this has a significant impact on the root exudate composition. In addition, we also analyzed the corresponding root and rhizosphere bacterial communities using 16S rRNA metabarcoding analysis. The advanced statistical analyses used to link the root exudate profile to the microbiome composition to find molecular mechanisms involved in microbiome recruitment by plants will be discussed.

P-437 Sting nematodes modify metabolomic profiles of host plants

PRESENTING AUTHOR: ***Denis Willett, Cornell University, United States***

CO-AUTHORS: ***Camila C. Filgueiras***

Many nematode endoparasites of plants have sophisticated means of modifying host metabolomes. Root knot and cyst nematodes can create sophisticated feeding cells that co-opt host cellular machinery to upregulate amino acid and sugar production. Given their lifecycle and long history of co-evolution with hosts, it makes some sense that endoparasitic plant nematodes can modify host metabolomes. Here, we show that, based on global metabolomic profiling, nematode ectoparasites of plants can also modify host plant metabolomes. Susceptible, moderately tolerant, and tolerant varieties displayed distinct metabolomic profiles in response to sting nematode feeding. Specifically, sting nematodes suppress amino acids in susceptible varieties. Upregulation of compounds linked to plant defense have negative impacts on sting nematode populations. Pipecolic acid, linked to systemic acquired resistance induction, seems to play a large role in protecting tolerant varieties from sting nematode feeding and could be targeted in breeding programs.

P-438 Metabolomic profiling defines key signals and antibiotics in drought-induced resistance to Cochliobolus heterostrophus

PRESENTING AUTHOR: ***Shawn Christensen, USDA-ARS, United States***

CO-AUTHORS: ***Casey A. Chamberlain, Charles Hunter, Anna Block***

In nature, plants are simultaneously challenged by multiple forms of biotic and abiotic stress that combine for diverse effects on crop production. Here we examined disease resistance under drought stress conditions and observed that the magnitude of fungal-elicited maize responses are quantitatively dependent on the duration of drought prior to inoculation (DPI). Comparative analysis of watered and drought stressed Cochliobolus heterostrophus-infected plants using metabolomic fingerprinting resulted in complete multivariate separation of the two global metabolomes with 2,367 significant molecular features. Among these features, phytohormones, amino acids, sugars, and oxylipins were strongly elicited by C. heterostrophus infection in plants undergoing DPI, including the drought responsive signal 12-oxo-phytodienoic acid. The phytohormone abscisic acid predictably increased in response to drought stress but was curiously suppressed in C. heterostrophus-infected tissues vs. damaged controls. Examination of ent-kaurene-, β-macrocarpene- and benzoxazinoid-related antibiotics in fungal-elicited tissues displayed drought-dependent increases in production over an eight-day time course. Transcript accumulation of the 1,3-β-glucanase PR6mb, pathogenesis-related 4 (PR4), and chitinase genes also demonstrated positive relationships between pathogen elicitation and the duration of DPI. Collectively, our results indicate that drought stress potentiates maize defense mechanisms, contributing to heightened resistance against the common maize pathogen C. heterostrophus.

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***AWARD WINNERS**

PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-439 Adaptive metabolomes: defense-related reprogramming in Sorghum bicolor

PRESENTING AUTHOR: ***Ian Dubery, University of Johannesburg, South Africa***
CO-AUTHORS: ***Charity Mareya, Fidele Tugizimana***

Background: Burkholderia andropogonis is the causal agent of bacterial leaf stripe, one of the three major bacterial diseases affecting Sorghum bicolor. However, the biochemical aspects of the pathophysiological host responses are not well understood. An untargeted metabolomics approach was designed to understand molecular mechanisms underlying S. bicolor–B. andropogonis interactions. At the 4-leaf stage two sorghum cultivars (NS 5511 and NS 5655), differing in disease susceptibility/resistance, were infected with B. andropogonis, and the metabolic changes monitored over time. Results: The NS 5511 cultivar displayed delayed signs of wilting and lesion progression compared to the NS 5655 cultivar, indicative of enhanced resistance. The metabolomics results identified statistically significant metabolites as biomarkers associated with the sorghum defence. These include the phytohormones salicylic acid, jasmonic acid and zeatin. Moreover, metabolic reprogramming in an array of chemically diverse metabolites that span a wide range of metabolic pathways was associated with the defense response. Signatory biomarkers included aromatic amino acids, shikimic acid, metabolites from the phenylpropanoid and flavonoid pathways, as well as fatty acids. Enhanced synthesis and accumulation of apigenin and derivatives thereof, was a prominent feature of the altered metabolomes. Conclusions: The analyses revealed an intricate and dynamic network metabolic pathways and metabolites comprisingthe sorghum defence arsenal towards B. andropogonis in establishing an enhanced defensive capacity in support of resistance and disease suppression.

P-440 Isotope assisted profiling of plant defense metabolites

PRESENTING AUTHOR: ***Maria Doppler, IFA-Tulln, University of Natural Resources and Life Sciences, Vienna (BOKU), Austria***
CO-AUTHORS: ***Christoph Bueschl, Bernhard Kluger, Rainer Schuhmacher***

Application of classical LC-MS based untargeted metabolomics workflows for the comparison of control and stress-treated plants results in the detection of numerous defense related metabolites. Although a large number of metabolites can be detected and classified to be of biological interest, the majority remains unknown or unidentified, which complicates biological interpretation. With our stable isotope labeling workflow we are not only able to effectively and reliably filter biologically relevant metabolites from background compounds, but also to obtain the number of labeling atoms (e.g. C and N) for all detected metabolites. Furthermore, the application of isotopically labeled tracers allows us to detect submetabolomes that only consist of metabolites descending from the applied tracer (i.e. a biological pathway). In the presented study, global-labeling as well as tracer-fate approaches were applied and combined in order to investigate the metabolic response of flowering wheat plants upon treatment with the mycotoxin deoxynivalenol. The detected phenylalanine-derived submetabolome consisted of 172 metabolites and up to 30% of them were classified as defense related. The substance class of hydroxycinnamic acid amides (HCAAs) turned out to be highly involved in the plants defense response as many of these metabolites' abundances were significantly increased under deoxynivalenol stress conditions. Based on these representative metabolites, the potential of SIL approaches for the annotation and identification of unknown metabolites is demonstrated for sum formula generation, database search and further characterization on the fullscan as well as on the MS2 level.

P-441 Comparative host-parasite and within-colony metabolomics: Cerithideopsis californica trematode guild

PRESENTING AUTHOR: ***Zdenek Kamenik, Institute of Microbiology, Czech Republic***
CO-AUTHORS: ***Daniel Metz, Ryan Hechinger, Martin Kurecka, Petr Marsik***

Despite the vast diversity of trematodes and their medical importance, we still lack basic knowledge about the biology of these parasites in their first intermediate host. The fact that these parasites are 'phenotype hijackers' (parasitic castrators), coupled with the recent discovery of social organization in some trematode species, makes the gastropod-trematode host-parasite system an attractive target for studies leveraging the power of comparative metabolomics with the experimental tractability of these organisms. In this study, we used the potamidid snail Cerithideopsis californica and selected trematodes from the diverse guild exploiting it to answer three questions using untargeted mass spectrometry-based metabolomics studies. Specifically, we investigated (1) mechanism by which the parasites directly sense changing conditions outside of their host; (2) the differences between the metabolomes of uninfected and infected snails; (3) the possibility that soldier trematodes release an alarm pheromone to recruit other soldiers to the defense of the colony.

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***AWARD WINNERS**

PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-442 Novel sources for Striga resistance: looking toward the rhizosphere

PRESENTING AUTHOR: ***Benjamin Thiombiano, University of Amsterdam, Netherlands***

CO-AUTHORS: ***Dorota Kawa, Aimee Walmsley, Hanna Vahldick, Siobhan Brady, Harro Bouwmeester***

Striga species are roots-parasitic plants widely distributed and able to infest rice, maize, sorghum and different other crops. S. hermonthica is considered the most serious worldwide parasitic weed, with an estimated affected area amounting to many millions of hectares. Aside of intercropping, fertilization, Striga low germinant genotype, soil microbiome has also been explored. Our preliminary data are suggesting that microbiome may lead to a decrease of Striga infestation rate. To better understand the mechanisms of the microbes mediated decrease of Striga infestation rate, evaluating the intercommunication between plant and the microbe through sorghum roots chemical composition and roots exudates chemical profile is needed. Here, we compared root exudates of sorghum under four different growing conditions (i.e. gamma radiated soil in presence and absence of Striga and non-gamma radiated soil in presence and absence of Striga). We analyzed roots and root exudates with a metabolomics approach and assessed phenotypic aspects such as Striga germination and attachment rate. We showed that a Striga attachment was reduced in presence of microbes. Using multivariate analysis, we observed a significant effect of the growing conditions on the root and roots exudate chemical composition. Further advanced statistical analyses will be used to link the roots and root exudate chemical composition to the striga infestation rate in order to find the mechanisms involved in microbiome microbes mediated decrease of Striga infestation rate.

P-443 Metabolomics studies of rhizosphere metabolites from 26 accession of Arabidopsis thaliana involve in plant interaction with rhizosphere microorganism

PRESENTING AUTHOR: ***Kourosh Hooshmand, Aarhus University, Denmark***

CO-AUTHORS: ***Enoch Narh Kudjordjie, Rumakanta Sapkota, Tong Shen, Oliver Fiehn, Mogens Nicolaisen, Inge S Fomsgaard***

Plants may regulate and selectively choose distinct members of a microbial community in the rhizosphere. The belowground plant-microbe association is facilitated by exudation of a wide range of bioactive chemical compounds from the root which may lead to indirect plant resistance against the potential biotic challenges. However, the underlying mechanism enabling the plants to shape its root microbiome structure has been poorly elucidated. In this study, 26 accessions of Arabidopsis thaliana (wild types) were grown in soil, harvested, lyophilized and extracted. The chemical composition of primary metabolites, secondary metabolites and lipids were determined by employing GC-TOFMS, HILIC-TripleTOF MS/MS, and reverse phase-Q Exactive HF mass spectrometer (CSH, lipidomics) respectively. GC-MS data was processed by BinBase and HILIC and CSH data were proceed by MS-DIAL_2.94 followed by post-processing with MS-FLO. The bacterial and fungal composition was determined by Illumina MiSeq sequencing technique. Carbohydrate, amino acids, and fatty acids were the main primary metabolites present in all the accessions. Carboxylic acids, organooxygen, and benzene derivatives were also predominated polar metabolites classes, as well as fatty acyls, glycerophospholipids, and sphingolipids were the core lipids in all the accession. Various statistical methods will be employed to identify the unique chemical substrates from the root of each A. thaliana accessions associated with regulation of distinct members of beneficial rhizosphere microbes which can lead us into a more sustainable plant production.

P-444 Metabolomics to exploit the primed immune system of tomato fruit

PRESENTING AUTHOR: ***Pierre Pétriacq, INRA & University of Bordeaux, France***

CO-AUTHORS: ***Estrella Luna, Amélie Flandin, Cédric Cassan, Chloé Chevanne, Camélia Feyrouse Kadiri, Yves Gibon, Pierre Pétriacq***

Tomato is a major crop suffering substantial yield losses from diseases as fruit decay at a postharvest level can claim up to 50% of the total production worldwide. Due to the environmental risks of fungicides, there is an increasing interest to exploit plant immunity through priming, which is an adaptive strategy that improves plant defensive capacity by stimulating induced mechanisms. Broad-spectrum defence priming can be induced by the compound ß-aminobutyric acid (BABA). In tomato plants, BABA induces resistance against various fungal and bacterial pathogens and different methods of application result in durable protection. Here, we examined whether treatment of tomato plants with BABA resulted in a durable induced resistance in tomato fruit against Botrytis cinerea, Phytophthora infestans and Pseudomonas syringae. Targeted and untargeted metabolomics were used to investigate the metabolic regulations that underpin priming of tomato fruit against pathogenic microbes that present different infection strategies. Assessment of infection showed a statistically significant reduction of disease symptoms in tomato fruit that originated from BABA-primed plants, thus confirming the broad effectiveness of BABA priming against various pathogens. Metabolomic analyses revealed major changes after BABA treatment and after infection. Remarkably, primed responses depended entirely on the type of infection, rather than showing a common fingerprint of BABA-induced priming. Altogether, our results demonstrate that metabolomics is particularly insightful towards a better understanding of defence priming in fruit. Further experiments are underway in order to identify key metabolites that mediate broad-spectrum BABA-induced priming in tomato fruit.

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***AWARD WINNERS**

PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-445 Metabolomic responses of tomato fruits and roots according to magnesium excess

PRESENTING AUTHOR: ***Min Cheol Kwon, Konkuk University, South Korea***

CO-AUTHORS: ***Eun Sung Jung, Yangmin X. Kim, Seulbi Lee, Choong Hwan Lee***

Green house cultivation uses excessive amounts of fertilizer due to the characteristics of intensive cultivation, causing nutrient salts that affect plant growth and development. Tomato is an important economic crop that is continually threatened by salt stress. To explore the effect of magnesium excess on metabolites of tomato plants, we performed non-targeted metabolite profiling. Tomato was cultivated under three different conditions, i.e. control, high magnesium stress (MgH), and extremely high magnesium stress (MgEH), and fruits and roots were harvested. In principal component analysis and heat map of fruit and root data, metabolite changing patterns of Mg stress showed different patterns in fruits and roots. Tomato fruit metabolic variations by Mg stress included increases of amino acids, flavonoids and amines and decreases of organic acid, sugar and lysophospholipids. Moreover, the highest anti-oxidant activity of tomato fruits was observed in MgEH group, which was positively correlated to the levels of flavonoids and amines. Also, the decrease of fresh weight per fruit caused by MgEH was positively related to the variations of organic acids and sugars. In tomato roots, MgEH decreased amino acids, organic acids and lysophospholipids. Taken together, our results revealed that tomato fruits and roots responded differently in response to magnesium excess in terms of amino acids, which increased in fruits and decreased in roots.

P-446 Stable isotope labelling and omics approaches to study the spatiotemporal regulation of diterpenoid resin acids biosynthesis in Norway spruce

PRESENTING AUTHOR: ***Monica Scognamiglio, Max Planck Institute for Chemical Ecology, Department of Biochemistry, Germany***

CO-AUTHORS: ***Felix Feistel, Erica Perreca, Christian Paetz, Jonathan Gershenzon, Axel Schmidt***

Many conifer species produce a complex mixture of terpenoids, known as resin, acting both as a mechanical and as a chemical barrier against herbivores and pathogens. Although the biosynthetic pathways of the resin components are known, the way some species like Norway spruce (Picea abies) regulate their biosynthesis and accumulation is still not well understood, yet very important in helping elucidating resin's biological and ecological significance. In order to gain new insights into the spatiotemporal regulation of the biosynthesis of diterpenoid resin acids (the main components of resin besides monoterpenes), P. abies saplings were placed in presence of 13CO2 during their growing season in an in-house built labelling chamber under controlled environmental conditions. The 13C enrichment was monitored over time. Thanks to a combination of metabolomics and target chemical analyses, it was possible to obtain information not only on the resin components' biosynthesis during the year, but also on the associated orchestration of plant metabolism. The results suggest that these compounds are synthesized and accumulated very early during the development of new growing tissues, while no turnover was observed in old tissues. The chemical composition of the resin mixture is highly regulated and organ/tissue specific. The identity and relative abundance of the different chemicals are very conserved in the branches, while they vary broadly and change over time in needles. In conclusion, the used experimental approach helped us to shed light on important aspects of resin biosynthesis and accumulation and showed a fine tuning of both phenomena in Norway spruce.

P-447 Elucidation of metabolic reprogramming that characterizes the plant growth promoting rhizobacteria priming of tomato plants

PRESENTING AUTHOR: ***Msizi Mhlongo, University of Johannesburg, South Africa***

CO-AUTHORS: ***Fidele Tugizimana, Lizelle Piater, Ian Dubery***

Plant growth promoting rhizobacteria (PGPR) are beneficial microbes found in the rhizosphere that directly or indirectly stimulate plant growth. Some PGPR can prime plants for enhanced defense against a broad range of pathogens and insect herbivores. In this study, four PGPR strains (Pseudomonas fluorescens N04, P. koreensis N19, Paenibacillus alvei T19 and Lysinibacillus sphaericus T22) were used to induce priming in Solanum lycopersicum (cv Moneymaker) plants. Seedlings were inoculated with an overnight culture of four PGPR strains and the different plant tissues (roots, stems and leaves) were harvested at 24 h and 48 h post-inoculation. Metabolites were extracted with methanol and analysed by UHPLC-MS/MS. Chemometrics methods were applied to mine the data and characterize the differential metabolic profiles induced by these PGPR strains. The results showed that the four PGPR strains induced a defence related metabolic reprogramming in tomato plants, characterized by changes in hydroxycinnamates, benzoates, flavonoids, amino acids and fatty acids. These metabolic alterations point to a preconditioned state that renders the plants primed for an enhanced defense responses. Thus, these results contribute to ongoing efforts in investigating and unravelling the biochemical processes that define the priming phenomenon.

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PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-448 Metabolomics and Machine Learning Techniques applied to investigate beneficial Plant-Bacteria Interactions

PRESENTING AUTHOR: *Lena Fragner, University of Vienna, Austria*

CO-AUTHORS: *Florian Schindler, Johannes Herpell, Anke Bellaire, Weimin Li, Xiaoliang Sun, Wolfram Weckwerth*

Endophytic non-pathogenic colonization of plant tissue by bacteria is a well-known and wide spread phenomenon, even expected to be the case for all angiosperms. Symbiotic plant-bacteria interactions comprise various levels of obligations and ecological benefits to at least one of the partners. The beneficial effects and functions for plants are manifold, including enhanced stress resistance, plant growth promotion or capacity for controlling plant-pathogens. In the present work, we focus on obligatory and constant symbioses occurring in the plant families Rubiaceae, Primulaceae and Dioscoreaceae. Highly specialized bacterial symbionts are mainly host-specific, often not cultivable and their absence can lead to dwarf phenotypes of the host-plants. Endophytic bacteria of leaves can be evenly distributed between the mesophyll cells or accumulated in specific leaf areas or in specialized structures. Most studies focused on genome and proteome analyses suggesting potential alterations of secondary metabolism caused by the presence of beneficial symbionts. However, detailed mechanisms and functions of these highly specialized mutualistic plant-bacteria symbioses are not yet fully understood. In the present study we investigate alterations in the metabolome of colonized leaf tissue. Primary and secondary metabolites were analyzed by GC-MS and LC-MS respectively, complemented with physiological and morphological data, and analyzed with machine learning techniques. Results indicate distinctive mechanisms of the symbiosis in investigated beneficial plant-bacteria interactions and will be discussed in detail.

P-449 Metabolic Profiling of Benzoxazinoids in Weed Suppressive and Early Vigour Wheat Genotypes

PRESENTING AUTHOR: *Leslie Ann Weston, Charles Sturt University, Australia*

CO-AUTHORS: *James M. Mwendwa, Paul A. Weston, Inge Fomsgaard, William B. Brown, Jeffrey D. Weidenhamer , Leslie A. Weston*

Wheat cultivar trials were conducted in moderate to low rainfall zones at Wagga Wagga and Condobolin NSW from 2014-2016. Crop and weed growth was monitored at tillering, vegetative, grain filling, harvest and post-harvest stages. Wheat roots, shoots, rhizosphere and bulk soils were collected over time for metabolomic profiling and extracted in methanol using an automated Buchi high pressure extractor while soil samples were extracted for 24h by rotary shaker. Non-targeted analysis by UPLC-ESI MS QToF (Agilent 6530) was performed and data analysed using Mass Profiler Professional (Agilent). Benzoxazinoids (BXs), including potent allelochemicals, were profiled using targeted analysis in negative ion mode while key bioactive phenoxazinones (microbially-produced APO, AAPO, AAMPO) were profiled in positive ion mode. Detection of up to 20 individual BXs including glycosides, lactones, hydroxamic acids and related microbial metabolites were noted. Both qualitative and quantitative differences in BXs were observed and were cultivar-, growth stage- and location-dependent. Plant part and rhizosphere location (distance from root) also impacted BX concentration. Microbially-produced metabolites with phytotoxic activity were detected at ecologically relevant concentrations in roots and rhizosphere soils. Metabolic profiling provided critical knowledge of seasonal impacts on wheat metabolism, as well as the biosynthesis and release of metabolites associated with weed suppression. Certain wheat cultivars, including heritage and recent commercial accessions, maintained high yield potential and were significantly more weed suppressive, depending on year and location, potentially due to their vigorous early growth habit and canopy architecture as well as the release of BXs and phenoxazinones into the rhizosphere over time.

P-450 The honey bee pollen diet investigated by MS and NMR based metabolomics

PRESENTING AUTHOR: *Nanna Hjort Vidkjaer, Aarhus University, Department of Agroecology, Denmark*

CO-AUTHORS: *Jane Ward, Per Kryger, Inge S. Fomsgaard*

Declining honey bee (Apis mellifera, hereafter bees) populations are concerning because bees are important pollinators of food crops. The decline is hypothesized to be driven by multiple factors (1), and bees face many stressors including pathogens, xenobiotics and changes in floral resources affecting their diet. Bees consume pollen containing essential nutrients and multitudes of bioactive plant secondary metabolites (PSMs). Recent findings demonstrate the potential of PSMs to affect bee health e.g. by reducing virus loads (2), but the PSM profile of pollen is sparsely investigated. Different floral resources are available in different environments and throughout the season, but limited knowledge exist on how such variations influences dietary composition. In a Danish field experiment, biweekly pollen samples (hive entrance and fermented pollen stores) were collected from four apiaries located in different environments (agricultural, urban, forest, and meadow). Chemical profiles of the two pollen types were generated by direct infusion ESI-MS, NMR, and HPLC-HRMS. Multivariate data analysis was subsequently used to explore seasonal and landscape variations in diet composition. The results clearly demonstrated differences in the composition of the pollen originating from the apiaries in the four environments. Within each environment, the pollen composition gradually changed throughout the season. Collectively, the results create a knowledge base for future studies of dietary effects on bee health and allows for novel insight into the chemical fate of PSMs upon hive storage and fermentation prior to consumption. 1. Goulson, D. et al. Science 2015, 347:1435. 2. Palmer-Young, E.C. et al. J Econ Entomol 2017, 110:1959.

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PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-451 From Field to Feature: A global workflow for metabolic fingerprinting in grassland communities

PRESENTING AUTHOR: ***Susanne Marr, IPB Halle, MLU, iDiv, Germany***

CO-AUTHORS: ***Jos Hageman, Kristian Peters, Nicole van Dam, Helge Bruelheide, Steffen Neumann***

Plants, grown in fields, face an undefined number of stresses. How plants respond to these factors, is often studied on the level of visible traits. However, changing communities and seasons will also be reflected in the metabolic fingerprint of a plant. Understanding those dynamics will help to unravel underlying mechanisms of ecosystem functions, such as herbivore resistance. One primary challenge that scientists face when bringing together high-throughput analytical tools with broad ecological questions is the lack of standardised methods that would allow for the automated analyses of metabolomic data within complex experimental designs. We aim to design a global workflow enabling large-scale ecological experiments including many diverse plant metabolomic profiles to be analysed and compared within one dataset. We analysed secondary metabolites – well known to play key-roles in plant defence strategies – in the aboveground tissue of 13 grassland species. In the Jena Experiment, plants were sampled from different communities, consisting of 1 to 8 different species. The plants were collected at four time-points across the growing season to capture seasonal differences. Our workflow enables the automated processing and analysis of raw data, obtained from UPLC-MS spectra, across all species and all treatments simultaneously. We use the number and composition of features – compound-fragments defined by their specific mass and retention time – to describe the metabolic fingerprint. The fingerprints identified each species across the different sampling conditions. On the species level, we found different responses to plant diversity and season, indicating species-specific strategies of adaptation to environmental changes.

P-452 Jasmonate-mediated tomato fruit growth under supplemental LED inter-lighting revealed by metabolomics

PRESENTING AUTHOR: ***Ivan Paponov, Research Scientist, Norway***

CO-AUTHORS: ***Martina Paponov, Dmitry Kechasov, Michel J. Verheul***

Fruit growth and the accumulation of primary compounds, which determine the final fruit quality, are driven by the supply of water, assimilates, and nutrients to the fruits through the phloem and xylem. Consequently, both shoot and root activities contribute to fruit growth. The supplemental LED inter-lighting can modulate both shoot and root activities; therefore, we suppose that this supplemental lighting also can modulate the relative growth rate of tomato fruits during the light and dark periods. How the diurnal rhythm of fruit growth is affected by supplemental LED inter-lighting treatment and the mechanisms of its regulation are unknown and needs further investigation. To address these questions, we investigated the diurnal response in term of fruit relative growth rate and we analyzed the metabolite composition of xylem sap. Diurnal analysis of fruit growth showed that fruits on plants receiving LED light grew quicker during the night than did fruits of control plants. This larger fruit growth during the night was related to an increased root pressure: the main source of night-time water and nutrient transport into the fruits. LED treatment also increased the levels of the phytohormone jasmonate in the xylem. Supplemental LED inter-lighting increased tomato fruit weight by increasing the total assimilates available for fruit growth and by enhancing root activity through increases in root pressure and water supply to support fruit growth during the night.

P-453 Metabolomic responses of Oryza sativa (rice) to high humidity conditions

PRESENTING AUTHOR: ***Venea Dara Daygon, The University of Queensland, Australia***

CO-AUTHORS: ***Melissa Fitzgerald***

Abiotic stresses induce a cascade of metabolic responses in plants to help in adaptation and survival during unfavourable conditions. In some cases, these changes affect the eating quality of food crops. In our previous study, untargeted profiling of volatile compounds in rice has identified indole as a major contributor to unpleasant odour in rice grains and its production is highly affected by environmental factors. To understand the mechanism of indole production, eight japonica rice varieties were grown in different levels of humidity during grain filling stage. Targeted analysis of indole was performed using GC-MS in the matured rice grains and metabolite profiling of auxins and indole derivatives was performed using SPE UHPLC-MS on different stages of rice development. We further compared the expression of candidate genes in varieties with varying levels of indole production. In this study, we demonstrate that high moisture and humidity during the mid to late grain filling stages enhances indole production in susceptible varieties. Indole expression in the matured grains could be a result of an alteration in the tryptophan-dependent biosynthesis of indole-3-acetic acid synthesis during stress conditions.

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P-454 Metabolomics of Brachypodium – fungal pathogen interaction

PRESENTING AUTHOR: ***Anna Piasecka, Institute of Plant Genetics, Polish Academy of Sciences, Poland***

CO-AUTHORS: ***Nicolas Jedrzejczak-Rey, Paweł Bednarek***

Brachypodium distachyon (Bd) is one of the model systems to study plant–pathogen interaction in grasses. However, the immune function of Bd secondary metabolites is until now unexplored. Therefore, metabolomics of Bd carries great potential to contribute to fundamental knowledge on plant immunity in cereal crops. Untargeted LC-MS approach in combination with MZmine2, MetaboAnalyst and MarVis cheminformatic tools enabled us to study secondary metabolism of Bd during infection with Parastaganospora nodorum, which is economically devastating fungal pathogen of cereals. Our analysis revealed that the number of differentially accumulated metabolites (DAMs) 72 hours post infection (hpi) was three times greater than 24 hpi. Compounds from biosynthetic pathways of indoles, phenylpropanoids, terpenes and polyamines have been identified as DAMs among thousands of detected signals. The most significant changes in metabolite accumulation were observed for serotonin and its derivatives. For instance, dehydrodimer of serotonin was one of the most highly accumulated metabolites 48 hpi. Conjugates of serotonin with p-coumaric and ferulic acids were also identified as DAMs. In addition, tryptamine, which is a precursor of serotonin, also increased its accumulation during infection. These results indicate a tight correlation of tryptophan and hydroxycinnamic acid metabolism with Bd immune response to fungal pathogens. This study was supported by the National Science Centre grants: Sonata 2015/17/D/NZ9/03347 and Sonata Bis 2012/07/E/NZ2/04098.

P-455 Tracking the metabolic fate of 13C-labeled plant-derived allelochemicals in the environment

PRESENTING AUTHOR: ***Eva Knoch, Gregor Mendel Institute of Molecular Plant Biology, Austria***

CO-AUTHORS: ***Niklas Shandry, Maria Doppler, Christoph Bueschl, Rainer Schuhmacher, Claude Becker***

Many plant species release specialized metabolites into the soil that inhibit germination, growth, or development of neighboring plants. These substances are collectively known as allelochemicals, and the related process as allelopathy. A key aspect of allelopathy is the post-release chemical dynamics of these compounds in soil: while some of them are converted to biologically more active forms, others are degraded and lose their toxicity. An important factor in the turnover of allelochemicals in soil is the rhizospheric microbiome, the community of microorganisms that live on and around the plant roots. To understand the metabolic processes that occur after release of the allelochemicals, we take a deconstructed approach that uses plant-derived stable-isotope-labeled allelochemicals and isolated bacterial communities. 13C-labeled allelochemicals are produced by growing plants on 13C-glucose and purified by preparative HPLC. Purified labeled and unlabeled allelochemicals are added to the growth media of defined bacterial communities; we then monitor their metabolic conversion and degradation products by LC-HRMS and will try to identify novel biotransformation products with MS/MS. The product patterns will be correlated with transcriptomes from the bacterial communities to reveal molecular mechanisms of allelochemical turnover by soil bacteria. Here we present our results to date, with focus on the workflow for production of 13C-labeled allelochemicals benzoxazinoids from rye and sorgoleone from sorghum. 13C-enrichment and isotopologue patterns of the target compounds produced in seedlings have been determined by LC-HRMS. We find that the benzoxazinoids are almost fully 13C-labeled, which is a prerequisite for the use of them in our downstream application.

P-456 Metabolomics profiling in patients with non-specific acute low back pain after Thai Herbal Sahatsatara Formula administration

PRESENTING AUTHOR: ***Manmas Vannabhum, Center of Applied Thai Traditional Medicine, Mahidol University, Thailand***

CO-AUTHORS: ***Pravit Akarasereenont, Manmas Vannabhum, Jantanee Wattanarangsan, Titchaphorn Palo, Kamontip Harnphadungkit, Pinpilai Jutasompakorn, Somruedee Chatsiricharoenkul***

Thai Herbal Sahatsatara Formula (STF) is in the Thailand National List of Herbal Medicinal Products for the relief of muscle pain and numbness on hands and feet. STF contains 21 herbal ingredients. The previous study showed that STF reduced pain in patients with low back pain (LBP). However, there are no identifying of related chemical compounds. The aim of this study is to investigate metabolites profiling, using liquid chromatography-time of flight mass spectrometry (LC-TOF MS), in patients with non-specific acute LBP after STF administration. Twenty-eight patients were received STF 1,350 mg, 3 times daily, for 7 days. Plasma samples were collected before STF administration on day 0, and after STF administration on day 1, 4 and 7. The results showed different metabolomics profile at day 1 and 4 when compared to day 0. Interestingly, the pattern of the chemical profile was closely when compared at day 7 to day 0. The most identified compounds were lipids such as lysophosphatidylethanolamine (LPE), palmitoleic acid, linoleate, myristic acid suggesting anti-inflammatory action of STF. Further study on the metabolomics-phenomics relationship will help to understand the mechanism of action of STF.

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POSTER SESSIONS 3 AND 4 – Wednesday and Thursday – all even number posters will be on display.

***AWARD WINNERS**

PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-457 Metabolomics as tool to study the role of semiochemicals in biotic plant interactions

PRESENTING AUTHOR: ***Roland Mumm, Wageningen Plant Research, Bioscience, Wageningen University and Research, Netherlands***
CO-AUTHORS: ***Ric C.H. de Vos, Robert D. Hall***

In nature, plants are surrounded by a huge diversity of organisms with which they form a complex network of antagonistic (e.g., pests and pathogens) and mutualistic (e.g., pollinators, beneficial microbes) interactions. These interactions are tuned by an intense chemical communication between the different players mediated by numerous volatile and non-volatile compounds, so called semiochemicals. In this presentation we will give examples of metabolomics approaches as a tool in research towards the role of different types of semiochemicals involved in the chemical communication of plants, microorganisms and insects. We have applied untargeted comprehensive GC-MS and LC-MS profiling for volatile and non-volatile metabolites to elucidate which semiochemicals are important for attracting and repelling of insect pest in contrasting crop species including cabbage, hop, and bean species. In bioassays and field trials we show that compounds that are also valued for their flavour properties likely also play a role in the plant resistance/susceptibility against enemies. In a plant microbial system we show that volatile compounds emitted by certain soil bacteria can have inhibitory effects on the growth of fungal plant pathogens but in turn can also promote the growth of the plant. Once we have identified these key metabolites we can exploit them in follow up research focused upon developing new strategies for improving crop production.

P-458 Metabolomic Approaches for the Identification of Flavonoids Associated with Weed Suppression in Sele

PRESENTING AUTHOR: ***Sajid Latif, Charles Sturt University, Australia***
CO-AUTHORS: ***Saliya Gurusinghe, Paul A. Weston, Jane C. Quinn, Leslie A Weston***

Incorporation of competitive pasture legumes in conservation agricultural systems provides a non-chemical alternative towards integrated and sustainable weed management strategies. While the in-field weed suppressive potential of annual pasture legumes has been previously described, the mechanism of interference with weeds has not been clearly elucidated. We therefore aimed to delineate the role of secondary metabolites present in pasture legumes through a series of studies to: 1) characterize key metabolites present in plant tissues, and the rhizosphere and 2) correlate their presence with weed suppressive properties. In vitro experimentations were conducted to assess the phytotoxic potential of selected annual pasture legumes and targeted and non-targeted metabolic profiling was performed to evaluate the abundance of key metabolites using UHPLC QTOF-MS. Methanolic extracts and dried residues of Biserrula pelecinus L. and Ornithopus compressus L., but not Trifolium vesiculosum Savi., exhibited marked phytotoxicity in a series of laboratory experiments. Metabolic profiling revealed that both foliar tissues and rhizosphere soils of annual pasture legumes possessed a high abundance of various flavonoids and their precursors. Chemometric analyses suggested a clear association of quercetin, isoquercetin, kaempferol, and kaempferol-7-O-glucoside with phytotoxicity and weed suppression under field conditions. Specifically, the abundance of quercetin and kaempferol was significantly higher in soils collected from established stands of biserrula and yellow serradella in contrast to arrowleaf, gland and subterranean clover. Both field and laboratory experimentation provided evidence for the role of annual legume-produced flavonoids in weed suppression in southern Australia and further insight into their localization and release in the soil rhizosphere.

P-459 Phytochemicals responsible for susceptibility to pest insects in forest species revealed by LC-MS based metabolomics approach

PRESENTING AUTHOR: ***Jasna Valentina Campos, University of Concepcion, Chile***
CO-AUTHORS: ***Sebastián Riquelme, Rosa Alzamora, Claudia Mardones, Rafael Rubilar, Andy J. Pérez.***

The forest industry is of great importance for the Chilean economy and the Eucalyptus plantations corresponds to 35% of total planted surface. The two most preferred species by the foresters are E. globulus and E. nitens, but these trees face large biotic stressors. Such is the case of Gonipterus platensis Marelli (Coleoptera: Curculionidae) commonly known as Eucalyptus weevil, which is a defoliator insect native from southeastern Australia that feed on growing Eucalyptus foliage. The most susceptible species to this pest in Chile is E. camaldulensis and E. globulus, whereas E. nitens has been reported for its ability to resist or avoid the attack, fact that has also been confirmed by our research group in the field. In the present work a new approach based on non-targeted reverse phase LC-MS metabolomics analysis was performed to establish a qualitative difference between metabolomes of E. globulus and E. nitens leaves, species with different susceptibility to the insect attack. The multivariate principal component analysis (PCA) and partial lest-squares discriminant analysis (PLS-DA) were performed to investigate the overall variation in the metabolome. The PLS-DA analysis showed the specific metabolites for each species where the most contributor to this separation highly correlated to E. nitens were STILBENOIDS and GALLATE derived compounds that explained the chemical difference with E. globulus, these secondary non-volatile metabolites can satisfactorily explain most of the variation between both species, consider them as potential biomarkers and playing an important role for the host selection by insect.

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***AWARD WINNERS**

PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-460 Metabolic profiling approach to understand the immune defense activity in date palm under biotic stress

PRESENTING AUTHOR: ***Resna Nishad, Qatar University, Qatar***

CO-AUTHORS: ***Talaat Ahmed***

The most devastating diseases of date palm are results of necrotrophic fungal pathogens, Ceratocystis radicicola is one among them. C.radicicola is found all over Arabian Peninsula, it is a causative agent of black scorch, root rot, trunk rot and rhizosis. Induced biosynthesis of primary and secondary metabolite is a plant basal immunity mechanism against fungal pathogen, thus in the current study we followed metabolic approaches to study the interaction between date palm and pathogenic fungi. As metabolite production and extraction is more convenient in suspension culture than the whole living plant, we established suitable date palm suspension culture to study the host-pathogen interaction. In order to understand whether same or distinct host defense pathway is operating in both in vitro as well as in planta system we compared the metabolic changes in callus and in planta after exposing plant cell to C.radicicola elicitor and mycelia. Fatty acid profiling was analyzed in response to fungal elicitor and mycelia in callus and leaf respectively by using GC-MS. The fatty acid production was different in callus from the leaf thus the response towards biotic stress was entirely different in both systems. Even though the response were different, the fatty acid synthetic pathway found to be leading SA mediated and JA mediated immune responses. It concludes that date palm suspension system can use for pant-microbe interaction study. This work was made possible by GSRA grant GSRA2-1-0608-14021 from the Qatar national research fund (a member of Qatar foundation).

P-461 In-depth metabolomic profiling reveals genome plasticity in the plant pathogen Fusarium poae

PRESENTING AUTHOR: ***David Overy, Agriculture and Agri-Food Canada, Canada***

CO-AUTHORS: ***Tom Witte, Amanda Sproule, Anne Hermans, Anne Johnston, Allen Xue, Linda Harris***

Fusarium poae (Peck) Wollenweber is cosmopolitan, occurring on a range of hosts and associated with Fusarium Head Blight (FHB) in cereals. European and Asian isolates of F. poae have been reported to produce type A and type B trichothecenes as well as beauvericin, cyclonerodiol, and enniatins, but little is known about the mycotoxigenic potential of Canadian isolates. Historically, mycotoxin production associated with F. poae is quite variable. Several researchers hypothesize that the variation in mycotoxin expression observed in F. poae results from the presence of supernumerary chromosomes and the abundance of transposable elements (TEs) within its genome. Supernumerary chromosomes act as evolutionary cradles for pathogen virulence factors and transposon facilitated translocations into core chromosomes can accelerate genome evolution and therefore present a considerable challenge towards a durable disease management strategy. In depth metabolomic profiling and genome sequencing was carried out on 46 monosporic Canadian F. poae isolates demonstrating a degree of chemotypic diversity. Metabolomic profiling revealed consistent production of multiple “core genome” associated metabolites including the emerging mycotoxins diacetoxyscirpenol and beauvericin. Of particular interest, strain specific production of new mycotoxins were also observed and linked with horizontal gene transfer through supernumerary insertions into the F. poae genome.

P-462 An untargeted lipidomics MS-based approach to identify bioactive compounds of different yeast species associated to Drosophila suzukii

PRESENTING AUTHOR: ***Flavia Bianchi, Laimburg Research Centre, Italy***

CO-AUTHORS: ***Urban Spitaler, Silvia Schmidt, Peter Robatscher, Daniela Eisenstecken***

The spotted wing drosophila, Drosophila suzukii (D. suzukii) is an invasive vinegar fly native to Southern Asia, that has spread across USA and Europe, causing considerable economic damage in agriculture to a wide variety of fruits. Previous studies revealed that yeasts are attractive to D. suzukii, and they can influence the fitness of many Drosophila species being a food source, and favouring larval development, fecundity and oviposition. Lipids play a fundamental role in the diet of Drosophila, since they are the major energy storage molecules in cells, and necessary for egg production. Insect fat body contributes to the development, metamorphosis, and reproduction of the flies. In nature, Drosophila feeds on yeast growing on fruit, and both yeasts and plants constitute a lipid source. Yeast food is enriched in phosphatidylinositols, in contrast, plant food contains mostly triacylglycerols, and a more heterogeneous composition of phospholipid classes. Within the project DROMYTAL the metabolic profile of eight selected yeast species putatively associated to D. suzukii have been explored to develop a lure, based on the association of attractant and phagostimulant yeasts and an insecticide. Intracellular non-polar metabolites have been extracted after inoculation in liquid media (potato dextrose broth) for 30 hours. An untargeted approach in reversed-phase liquid chromatography–quadrupole/time-of-flight mass spectrometry (RPLC-QTOFMS) was used to detect diverse compound classes. Clear differences could be found among yeast species. Overall, about 90 distinct intracellular lipids have been annotated and/or identified, for further investigating which compounds play a bioactive role for the fitness of the insect.

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***AWARD WINNERS**

PLANT, FOOD, ENVIRONMENTAL AND MICROBIAL

P-463	**Metabolomics reveals key chemical responses and susceptibilities in commercial forest species to infestation by pest insects introduced in Chile**

PRESENTING AUTHOR: ***Andy Pérez, Department of Instrumental Analysis, Faculty of Pharmacy, University of Concepcion, Chile***

CO-AUTHORS: ***Valentina Campos, Sebastián Riquelme, Claudia Mardones, Rosa Alzamora, Rafael Rubilar***

Forest industry is of great importance for the Chilean economy, with annual exports over US$ 5000 million since 2011 and planted surface around 2.4 million ha. Pinus radiata and Eucalyptus (mainly E. globulus and E. nitens) are the preferred species by forest companies, covering the largest plantation area in Chile. However, P. radiata as Eucalyptus species currently face biotic stressors that significantly threaten profits of this economic activity. One of these is the woodwasp Sirex noctilio F., considered as the most damaging invasive pest in Southern Hemisphere Pinus plantations, and the eucalyptus defoliator, Gonipterus platensis M. These are insects under official control in Chile declared by the Chilean Livestock and Agricultural Service (SAG). Here, we describe the LC-MS based metabolomics analysis on P. radiata wood, bark and needles from resistance and susceptible trees, aimed to distinguish defensive or resistance mechanisms against S. noctilio. Among revealed results, down-regulation for biosynthesis of taxifolin (dihydroquercetin) and derived of citric acid in the bark of infested trees, seems to be the most significant. Similarly, metabolites responsible for the different susceptibility between E. globulus and E. nitens to the defoliator G. platensis were distinguished. A combination of stibenoids and gallate derived metabolites present in the leaves of the less susceptible species was suggested as potentially exerting a feeding deterrent effect, opening a new possibility for the environmental friendly control of this pest. These results may contribute to uncover genetic basis for resistance mechanisms that could be harnessed in a resistance breeding program of such species.

P-464*	**Metabolomics profiling of meconium using LC/HRMS**

PRESENTING AUTHOR: ***Nihel BEKHTI, UMR CEA-INRA, France***

CO-AUTHORS: ***Florence Castelli, Estelle Paris, Blanche Guillon, Christophe Junot, François Fenaille, Karine Adel-Patient***

Some factors of exposure in pregnant women such as diet, living area, drugs are simultaneously transferred to the fetus and may affect its maturation and later, the child health development. These in utero transmissions could be observed through meconium analysis. Actually, meconium is the earliest newborn stools, it begins forming from the second month of pregnancy and accumulate until birth. This matrix has the advantage to integrate a large period of exposure. In the literature, meconium composition has been mostly studied using targeted methods focusing on a given family of compounds (ex pesticides, illicit drugs or commonly prescribed medicines). In this work, we aim to provide an individual mapping of the meconium composition, using non-targeted metabolomics analysis. We first developed and optimized the analysis workflow thanks to two meconium samples collected days 1 and 2 post-partum. High detection sensitivity and method robustness have been reached thanks to thoroughly optimized sample preparation, involving a pre-step of freeze-drying followed by a solvent-assisted metabolic extraction. This step is monitored by a liquid chromatography coupled to high resolution mass spectrometry (LC/HRMS) analysis, using a Q-Exactive instrument. Raw data were further processed using the Workflow4Metabolomics infrastructure. We successfully annotated up to 200 metabolites with a high level of identification, which represents the most exhaustive description of human meconium to date. This workflow is now being applied to a meconium cohort collected from 11 children, from birth to days 2-3 post-partum. This analysis will allow to evaluate the meconium composition evolution, and to assess inter-individual variability.

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*AWARD WINNERS

TECHNOLOGY

P-465 High Throughput Polar Metabolite Analyte Metabolomic Phenotyping of Large Cohort Epidemiological Studies Using Ion Mobility Enabled LC/MS

PRESENTING AUTHOR: ***Robert Plumb, Imperial College London, United Kingdom***

CO-AUTHORS: ***Robert Plumb, Adam King***

As global life-styles change we are seeing increasing cases of obesity, diabetes, and mental health issues. This not only affects a person's quality of life but also places increased strain on the health-care systems to provide the right treatment whilst managing costs closely. Metabolic Profiling of large cohorts offers a valuable and unique insight into the underlying biochemistry of diseases as well as the patient's individual biochemistry "phenotype', diet, health status, age and stress. To deliver this information the analytical data generated in processed via a variety of chemometric modelling and analysis methodologies to deliver the relevant biochemical information. These chemometric platforms employed vary from simple multivariant analysis to highly complex model based analysis and is presented in a format ready for interpretation by medics. On of the major challenges is the identification of putitive biomarkers. Recent advancements in ion mobility MS and in-silico CCS prediction offers the opportunity to revolutionize biomarker identification both in terms of speed and accuracy. In this presentation we will discuss the development of rapid exploratory LC/IMS/MS analytical platforms as well as a detailed discussion on the workflow, validation, reporting and decision-making process. The presentation will cover the development and validation of the "discovery' screening methods for polar, non-polar metabolites using LC/MS methodology, as well as describe the use of Ion Mobility Mass Spectrometry to enhance data quality.

P-466 Using the Retention Time Prediction Method for Choline Plasmalogens Identification by Liquid Chromatography-Mass Spectrometry

PRESENTING AUTHOR: ***Dave Lee, National Taiwan University, Taiwan***

CO-AUTHORS: ***Sung-Chun Tang, Ching-Hua Kuo***

Choline plasmalogens (P-PC) are special subclass of phosphatidylcholine (PC) which compose of a vinyl-ester bond at the sn-1 position of the glycerol backbone. Abnormal P-PC levels have been found associated with many diseases, such as neurological disorders and oxidative stress. Previous MS methods showed limitations on distinguishing P-PC and alkyl-PC (O-PC), as these PC subclasses share the same molecular formula and MS2 fragment ions. Herein, we developed a UHPLC-QTOF-MS method combined with the dynamic MRM (dMRM) method by UHPLC-QqQ-MS/MS for measuring PC molecule species. To identify P-PC and O-PC, we developed a retention time (RT) prediction method by establishing the linear correlation between prediction parameters considering the XlogP values and RT. The RT prediction method was applied to predicted 205 of the plasma PCs, including 33 P-PC and 51 O-PC, and over 60% of the identified PCs showed less than 0.3 minutes errors compared to experimental RT values confirmed by the fragmentation information. This proposed method was applied to study oxygen-glucose deprived (OGD) induced neuron injury. The results revealed PC(O-16:1/20:3) and PC(P-16:0/20:3) with identical formula, but different in the sn-1 structure were regulated in a distinct direction after OGD treatment. With the power of the RT prediction method to provide more structure information for PCs especially on P-PC and O-PC, the biological functions of these PCs could be uncovered. It is anticipated to apply this RT prediction method to explain unknown physiological mechanisms involving PC dysregulation and discover PCs as disease markers.

P-468 MetaboShiny - identify each mass, en masse

PRESENTING AUTHOR: ***Joanna Wolthuis, UMC Utrecht, Netherlands***

Analyzing untargeted mass spectrometry data involves investigating hundreds to thousands of metabolite peaks. This is a major challenge in direct infusion, where samples are introduced into the spectrometer with no column beforehand. Various analytical tools have been created to stand up to this challenge, but are often limited in identifying compounds. To analyze untargeted metabolomics data, one needs to match molecular candidates to many mass/change (m/z) values. To streamline this process we are developing the R tool MetaboShiny. It simplifies discovering m/z of interest from a pool of m/z values in an attractive and interactive interface. MetaboShiny features interactive plots and tables, with a tight integration of a fast SQLITE database backend. Available databases (16 total) include the HMDB, ChEBI, and KEGG. MetaboShiny generates adduct and isotope variants based on user preference. Altogether, the databases encompass over 100 million m/z values. MetaboShiny does normalization and statistics with the MetaboAnalystR package, such as T-tests, PCA, PLS-DA and fold-change analysis, to heatmaps and over 40 machine learning methods. Venn diagrams summarize results, identifying metabolites that are significant in multiple analyses. Users click through results and link significant m/z values to databases. MetaboShiny displays compound descriptors and PubMed abstract search results to enhance exploration. If no matches are found, MetaboShiny predicts a molecular formula from the m/z value, and searches PubChem for known compounds matching the predicted formula. We are implementing network/pathway-based and cheminformatics-based compound identification and neural network adduct formation prediction.

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*AWARD WINNERS

TECHNOLOGY

P-469 Using Multi-omics Approaches to Maximize Beef Production

PRESENTING AUTHOR: ***Aidin Foroutan, Department of Agricultural Food and Nutritional Science, University of Alberta, Canada***

CO-AUTHORS: ***Aidin Foroutan, Carolyn Fitzsimmons, Leluo Guan, Rupasri Mandal, David S. Wishart***

Over 40% of the cost of beef production is impacted by feed efficiency. Reproductive performance of beef cattle also has a significant impact on the cost of production. Hence, decreasing the cost of production along with improving carcass yield and quality will benefit the beef industry. Maximizing production efficiency of beef cattle requires not only genetic selection to maximize feeding efficiency (i.e. residual feed intake - RFI) but also adequate nutrition throughout all stages of growth and development - even during gestation. Nutrient restriction during gestation has been shown to negatively affect post-natal growth and development as well as fertility of the offspring. This, when combined with RFI, may affect progeny traits. Therefore, we decided to conduct a comprehensive multi-omics study to investigate the impacts of pre-natal nutrition and RFI selection on the metabolism and fertility parameters in Angus bulls. Four different tissues (Longissimus thoracis muscle, semimembranosus muscle, liver, testis) and three biofluids (serum, semen, rumen content) were analyzed. Epigenetics, transcriptomics, and metabolomics experiments identified 891, 4, and 47 candidate biomarkers, respectively, that were associated with RFI as well as pathways such as cellular growth and proliferation, embryonic development, organ morphology, and connective tissue development. These markers have been integrated through a variety of novel bioinformatic techniques to reveal key biological pathways affected by RFI selection and pre-natal nutrition in Angus bulls. If these gene/metabolite biomarkers and corresponding pathways are validated in a larger animal population, they could potentially be used in breeding programs to select for superior animals.

P-470 Automated analysis of large-scale NMR data generates metabolomic signatures and links them to candidate compounds and genes

PRESENTING AUTHOR: ***Sven Bergmann, University of Lausanne, Switzerland***

CO-AUTHORS: ***Bita Khalili, Mattia Tomasoni, Mirjam Mattei, Roger Mallol Parera, Reyhan Sonmez, Daniel Krefl, Rico Rueedi***

Applying genome-wide association studies (GWAS) to metabolomics associates metabolites with genotypic variants. We observed that the effect of a genetic variant on the concentration of a metabolite often translates into associations with all or many features of the metabolite NMR spectrum. The set of association scores with all measured features provides a pseudospectrum across the full range of ppm covered by 1H NMR spectra. The challenge is then to identify the metabolite underlying the most significant associations. To this end we developed the analysis tool metabomatching, which takes as input a pseudospectrum and a collection of reference spectra for individual metabolites such as found in HMDB. We first demonstrate that metabomatching works well to prioritize the most likely metabolite candidates for pseudospectra derived from metabolic feature association with genotypes (Rueedi et al. PLoS Genetics 2014 and PLoS Computational Biology, 2017). Next we show that the metabomatching methodology can also be used for identifying metabolites that vary across large collection of samples without the need for any external variables associated with this variation. We investigate three methods that identify co-varying spectral features within large-scale NMR data. Specifically, we compare Principal Component Analysis, the Iterative Signature Algorithm and averaging correlation profiles inspired by the STOCSY approach. For each method, we devise a principled way for processing their output into pseudospectra. We then evaluate systematically in which cases metabomatching provides strong evidence for matching metabolites, and assess to what extent the three methods provide consistent or complementary output.

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*AWARD WINNERS

TECHNOLOGY

P-472 Integration of metabolomics and transcriptomics to assess the effects of environmental pollutants

PRESENTING AUTHOR: ***Joaquim Jaumot, IDAEA-CSIC, Spain***

CO-AUTHORS: ***Elena Ortiz***

In the framework of the omics sciences, there is a current trend towards the integration of information coming from different omics levels. Advanced processing tools and novel data fusion strategies have been developed to gather information allowing a better understanding of the underlying biological processes. However, multiomics data coming from multiple sources is heterogeneous, and the mining of information using these data fusion approaches remains a challenge. Different strategies have been proposed to perform this data fusion. In general, three approaches can be identified depending on the processing phase at which data is fused: 1) low-level fusion combines raw data to generate a new fused data to be analyzed, 2) mid-level fusion involves joining subsets of relevant variables from different datasets, and 3) high-level fusion just merges results from the independent analysis for a combined interpretation. In this work, the effects of bisphenol A (BPA) in regulatory pathways of zebrafish embryos is evaluated by using a non-targeted LC-MS based metabolomics analysis and a high-throughput RNA sequencing (RNA-Seq). Independent metabolomics and transcriptomics results revealed a similar set of altered pathways despite the different number of detected potential biomarkers. Then, different integration approaches have been employed to deepen knowledge about the affected metabolomic pathways (mainly signaling pathways) in an attempt to decipher possible divergences in the information provided by the two omic levels.

P-473 The Power of MS/MSALL Acquisition for High-Throughput Metabolomics Studies

PRESENTING AUTHOR: ***Mariateresa Maldini, SCIEX, Italy***

CO-AUTHORS: ***Eva Duchoslav, Cyrus Papan, Khatereh Motamedchaboki***

Flow Injection Analysis using an electrospray source gives a simple technique maximizing analytical throughput and yet allowing metabolite separation by m/z in complex samples by using a data independent MS/MSALL acquisition approach.This technique combined with tailored data processing methods built from the accurate mass metabolite spectral libraries allows fast qualitative and quantitative sample profiling.The MS/MSALL acquisition, a sequential precursor ion fragmentation acquisition technique,with metabolome–tailored precursor mass defect captured high resolution MS and MS/MS for every mass.MultiQuant processing method was constructed using the metabolite experimental MS/MS spectra from the Accurate Mass Metabolite Spectral Library (SCIEX).To minimize the effect of mixed MS/MS spectra, MS/MS responses for series of up to 5 unique fragments typically including unfragmented molecular ion, combined with a response in TOF MS were extracted for each target metabolite. Calibration curves were constructed for set of 35 representative compounds. For example, in the positive ion mode the % CV for Tryptamine replicate measurements was better than 15% and the linear dynamic range was greater than 200 (coefficient of determination r2 > 0.95) based on groups of 3 independent fragments. More than 70% of spiked compounds in the standard mix and more than 50% of spiked compounds in the urine matrix were identified,and supplement the identifications with additional compound classifications. These preliminary data complemented with statistical analysis illustrate a potential of the MS/MSALL approach for high-throughput metabolomics studies where a fast,semi-quantitative profiling approach is to be combined with a confidence in compound identity and a potential for identification of unexpected secondary metabolites.

P-474 Novel visual analytics platforms for multi-omics integration and network visualization

PRESENTING AUTHOR: ***Guangyan Zhou, McGill University, Canada***

CO-AUTHORS: ***Huiting Ou, Guangyan Zhou, Jianguo Xia***

Multi-omics integration is emerging as a powerful strategy to simultaneously analyze and interpret multiple datasets from different omics technologies. It aims at gaining systems-level understanding of different disease states and other experimental conditions by revealing novel mechanistic insights of molecular pathways involved. There is an urgent need for user-friendly bioinformatics tools dedicated for multi-omics network integration and visualization. To address these challenges, we are actively developing two web-based platforms - OmicsAnalyst and OmicsNet to support statistics-based integration and network-based integration, respectively. OmicsAnalyst aims at facilitating the access to multivariate methods including O2PLS, Procrustes analysis, multiblock PCA and PLS. In addition, it uses advanced visual analytics techniques including 3D scatter plot and interactive heatmap, to deconvolute the complex outputs of these methods. OmicsNet enables users to create and merge different types of biological networks and visualize using an innovative WebGL-based system in 3D space. Users can query known interactions between seven different types of elements including SNPs, genes/proteins, TFs, miRNAs, metabolites, mass peaks and phenotypes, within the context of current knowledge.

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*AWARD WINNERS

TECHNOLOGY

P-475 Matching Untargeted Liquid Chromatography - Mass Spectrometry Features Across Multiple Cohorts: Finding the Same Needles in Several Haystacks Via Networks

PRESENTING AUTHOR: *Rui Climaco Pinto, Imperial College London, United Kingdom*

CO-AUTHORS: *Ibrahim Karaman, Matt R. Lewis, Jenny Hällqvist, Manuja Kaluarachchi, Gonçalo Graça, Elena Chekmeneva, Mohammad Arfan Ikram, Abbas Dehghan, Paul Elliott, Ioanna Tzoulaki, David Herrington, Timothy Ebbels*

In untargeted metabolomics, accurate within-dataset feature matching is aided by accessing large amounts of chromatographic/spectral information in each sample. In contrast, when matching features from multiple cohorts/batches that used the same analytical method but were acquired and peak-picked separately, only limited information is available - often only retention time and m/z (RT-MZ) medians. We propose a new cross-cohort matching method which uses the RT-MZ median values of each feature (plus optionally feature intensity and other feature-quality measures). Initially matches within manually-defined RT-MZ thresholds between all one-to-one dataset combinations are detected. In the next - key - step it builds a network of features (nodes) based on those matches (edges). Only matches in subnetworks (connected components) in which all nodes are connected to all other nodes (maximal cliques) are accepted, which removes features that matched by chance. As some features may present multiple matching possibilities, a third step using RT-MZ differences to define a match-quality score is then used to decide the best single matches. We illustrate the method's performance by matching thousands of features from large studies of serum samples and inspecting the matching accuracy of hundreds of manually annotated metabolites, and by looking at several feature-quality metrics. The method is not computationally intensive – e.g. matching 3 cohorts, each with 1000s of features, takes less than a minute on a typical desktop machine. The new approach addresses a key problem in metabolomics studies and promises to make analysis of large untargeted multi-cohort data sets a viable option.

P-476 On-demand construction of deep reference libraries and how they improve compound ID

PRESENTING AUTHOR: *Michal Raab, HighChem Ltd., Slovakia(Slovak Republic)*

CO-AUTHORS: *Jakub Mezey, Samuel Benkovič, Melissa Montoya, Tim Stratton, Robert Mistrík*

Compound ID through library searching is arguably the leading application in LC/MS, yet it unconditionally relies on the quality and coverage of available spectral libraries. However, experimental diversity and hard-to-reproduce fragmentation drives the volume of the LC/MS libraries to grow disproportionately compared to GC/MS, posing a challenge for data processing, management and searching applications. Here we describe an integrated pipeline for high-throughput acquisition of reference compounds and semi-supervised curation utilizing dedicated software for automated instrument control and scalable modular microservices-based architecture for quality control, curation, annotation and continuous delivery of library records. This system has been employed to handle the reference standards in the mzCloud library, allowing to expand the coverage of unique compounds by 7.500 in two months. HRAM-MS/MS data was acquired for reference standards by nanoinfusion using an automated tool that acquired fragmentation data at increasing NCE (Normalized Collision Energy) levels of 10-200 in increments of 10. Intelligent controlling software was deployed for batch-like data dependent MSn acquisition of reference compounds. A collection of data processing services has been built using a docker-based microservice platform including feature detection/extraction, noise filtering, annotation, fragment prediction and mass error correction. Additionally, separate quality control services are employed for real-time data inspection. A web-based frontend is used to monitor and control the processing queue, applying a sequence of the processing services from one of the pre-defined profiles individually for each spectral reference dataset, and to provide feedback through quality control indicators.

P-477 Integrating Polygenic Risk Scores and Metabolite Quantitative Trait Loci to Infer Dysregulated Mechanisms in Rheumatoid Arthritis Subtypes in Women

PRESENTING AUTHOR: *Su Chu, Brigham and Women's Hospital and Harvard Medical School, United States*

CO-AUTHORS: *Jing Cui, Jeffrey A. Sparks, Bing Lu, Clary Clish, Jessica Lasky-Su, Elizabeth Karlson, Karen Costenbader*

Background: Rheumatoid arthritis polygenic risk scores (RA-PRS) improve RA risk prediction, but the added predictive value over clinical variables is modest. Several human leucocyte antigen (HLA) haplotypes are strongly related to seropositive RA, a severe subtype. Recently, we identified several metabolites associated with RA risk. Integrating RA-PRS and metabolomics may provide insight into RA pathogenesis. Methods: Plasma samples from 254 pre-RA cases in the Nurses' Health Studies were analyzed using untargeted liquid-chromatography mass-spectrometry (360 unique metabolites after quality control). PRS comprised 1) non-HLA 93 single nucleotide variants (PRS93), and 2) HLA haplotypes (HLA-PRS) previously associated with RA risk. Using ordinary least squares, associations between both PRS93 and HLA-PRS and individual metabolites were tested to identify RA-related metabolite quantitative trait loci (metaboQTLs). Interaction models assessed effect modification by RA serostatus. Results: After multiple comparison adjustment using the pooled local index of significance, no PRS93 metaboQTLs were found; however, 27 RA HLA-PRS metaboQTLs were identified, including those involved in branched chain amino acid and polyamine metabolism: C2 carnitine (β=0.120; padj=0.018), C3:carnitine (β=0.122; padj=0.007), C5:1 carnitine (β=0.120; padj=0.016), 4-acetamidobutanoate (β=0.134; padj=0.027), and N-acetylputrescine (β=0.119; padj=0.011). Two findings suggesting effect modification of metabolite levels by seropositivity were observed in RA-HLA metaboQTLs: C5:1 carnitine (p-interaction=0.068) and arecaidine (p-interaction=0.077). Conclusions: We identified several metaboQTLs of HLA haplotypes in pre-RA, but none for PRS93. Evidence to support effect modification of the HLA-PRS and metabolite association by RA serostatus was identified for one acylcarnitine and one alkaloid derivative. However, further validation is required.

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TECHNOLOGY

P-478 Food Metabolome Repository: A database for cross-sample specificity-based peak prioritization in untargeted metabolomics

PRESENTING AUTHOR: ***Nozomu Sakurai, National Institute of Genetics, Japan***

CO-AUTHORS: ***Kunihiro Suda***

The substantial issue in mass spectrometry (MS)-based untargeted metabolomics is a lack of information by which we can prioritize the candidate peaks for further detailed investigation, rather than the accuracy of metabolite prediction/annotation tools. The major bottleneck in untargeted metabolomics is an annotation of compound peaks largely due to limited availability of authentic standards for identification. A lot of computational tools and databases have been developed so far for prediction of metabolites using accurate mass value, pattern of isotopic ions, and MS/MS spectra. However, the results from most of them are the candidate lists with some evaluation scores and the candidate with the highest score is not always true. Therefore, we have to investigate the validity of the prediction results one-by-one based on the other information such as literature knowledge of the occurrence of the candidate compound in nature. However, the occurrence of unknown peaks is not available because of the lack of databases by which peaks from a wide variety of samples can be compared and searched. To tackle this, we developed Food Metabolome Repository (http://metabolites.in/foods). A large variety of compounds is expected in foods because a lot of biological species are included and they can be processed by heating and fermenting. Untargeted metabolome data obtained from 222 foods using reversed-phase liquid chromatography-high resolution MS with electrospray ionization (ESI) positive and negative modes are available. The searching and acquiring functions can be integrated into other computational tools for automatic and large-scale processing via application programming interfaces (APIs).

P-479 Toward a high-quality non-targeted analysis of large metabolomics data

PRESENTING AUTHOR: ***Masahiro Sugimoto, Keio University, Japan***

CO-AUTHORS: ***Masahiro Sugimoto, Rintaro Saito, Tomoyoshi Soga, Masaru Tomita, Toru Takebayashi***

Toward a high-quality non-targeted analysis of large metabolomics data - Masahiro Sugimoto, Rintaro Saito, Tomoyoshi Soga, Masaru Tomita, Toru Takebayashi. Metabolomics cohort studies are becoming more widespread. Data processing and correcting data to remove unexpected biases are important for quality controls (QCs). We have conducted the Tsuruoka Metabolomics Cohort Study enrolling 11,002 community-dwelling adults in Japan. Capillary electrophoresis-mass spectrometry (CE-MS) has been used for the identification and quantification of hydrophilic metabolites in human plasma. Here we developed processing tools to analyze these data. First, we have developed MasterHands, a Java-based GUI software which is capable of versatile data analysis of CE-MS data. We upgraded this software to have an application programmable interface for Python language. We prepared two types of programs, one for non-targeted and the other for targeted analyses. Firstly, we conducted a non-targeted analysis of QC sample and analysts conducted curation of analyzed data in order to optimize analytical parameters for individual peaks. The characteristics of curated peaks were used for training data for the subsequent analysis. The other samples were analyzed using targeted analysis program and the curation of each peak integration are automatically adjusted following to the training data. These two-step analyses enable high-quality data processing even in the large scale datasets. Here, we show the algorithm and the performance of the processed data.

P-480* A genome-wide association study of circulating plasma metabolite levels identifies differences by sex and suggests that metabolites represent polygenic traits

PRESENTING AUTHOR: ***Oana Zeleznik, Harvard Medical School and Brigham and Women's Hospital, United States***

CO-AUTHORS: ***Oana A. Zeleznik, Xia Jiang, Rachel Kelly, Marta Guasch, Constance Turman, Jessica Lasky-Su, Clary B. Clish, A. Heather Eliassen, Peter Kraft***

Background: Genome-wide association studies (GWAS) have identified single nucleotide polymorphisms (SNPs) associated with disease risk but the mechanisms driving these associations remain largely unknown. Understanding the effects of SNPs on the metabolome of healthy individuals may elucidate the mechanisms linking SNPs to disease. We performed a GWAS of circulating plasma metabolomics (metaboQTLs) in 4390 participants of Nurses' Health Studies (NHS), NHS II and Health Professionals Follow-up Study (HPFS). We also performed a genome-wide screen for metaboQTLs showing differences by sex. Methods: Linear regression of metabolite levels and genotype, adjusted for age, cohort, fasting status and the first four genotype principal components was used to identify metaboQTLs. Cochran's Q-test was used to identify metaboQTLs showing heterogeneity by sex. Results: Seventy-four metabolites were associated with 93 independent SNPs ($p<2.3E-10$). Individual SNPs were associated with 1-21 metabolites and individual metabolites with 1-6 SNPs. The top identified metaboQTL ($p=5.37E-248$) was between a SNP next to carbamoyl-phosphate synthase 1 (CPS1) and glycine. CPS1 encodes an enzyme known to directly modify glycine. Fifteen of the 93 independent SNPs represent new metaboQTLs (LDr2<0.5 with published metaboQTLs). Twenty-three metaboQTLs showed genome-wide significant heterogeneity by sex. The metaboQTL of a SNP on the gene fatty acids dehydrogenase 2 (FADS2) and cholesteryl ester C20:4 showed strongest evidence for sex differences ($p=4.91E-39$). Conclusion: Multiple SNPs were associated with circulating metabolite levels. We identified new associations and also validated previously published results. Several metaboQTLs showed heterogeneity by sex. Some metabolites were associated with multiple variants, suggesting that metabolites may represent polygenic traits.

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TECHNOLOGY

P-481 MsCompare: An Untargeted GC/MS Metabolomics Platform for Quality Control, Precise Deconvolution and Data Analysis

PRESENTING AUTHOR: ***Marco Ruijken, MsMetrix, Netherlands***

A GC/MS workflow for Metabolomics includes a number of distinct steps: Experimental Design, Sampling, Sample Preparation, Data Analysis, Identification and Data Interpretation. The MsCompare platform includes all tools to properly control each step in this workflow. One of the key issues in this field is the precise and sensitive detection of all components present in a series of samples. GC/MS deconvolution remains by far the most difficult step for low level components, especially when highly similar co-eluting or nearly co-eluting compounds are present. In these cases, precise GC/MS Peak Detection and Deconvolution is necessary with minimal user interference. Proper deconvolution also allows for correct identification of all components. Another problematic area in GC/MS Metabolomics studies might be the proper alignment of chromatograms before the actual data processing starts. Depending on the application, we often see individual components in GC/MS having bad peaks shapes or bad reproducibility regarding retention times. MsCompare contains a number of alignment algorithms to correct for this behavior. Data analysis in MsCompare comprises both Univariate and Multivariate analysis methods like PCA, PLS-DA, Clustering etc. However, it will be shown that for many cases, due to the high selectivity of GC/MS, univariate analysis methods are adequate in solving the main questions. Examples from a number of different studies (small and large) will be given, showing an overview of the workflow and implemented tools.

P-482 A statistical approach to classification of mechanistic computational models of Parkinson's Disease

PRESENTING AUTHOR: ***Lalithasushma Chakravadhanula, LIACS, Netherlands***

CO-AUTHORS: ***Agnieszka Wegrzyn, German Preciat, Alissa Schurink, Edinson Lucumi, Michael Emmerich, Ronan M.T. Fleming***

Parkinson's Disease (PD) is a progressive neurodegenerative disorder. It is the second most common disease that affects the central nervous system and affects 7-10 million people worldwide. PD manifests with both motor symptoms such as tremors, rigidity, and non-motor symptoms like depression and fatigue. Although the pathogenesis of PD remains a puzzle, several genetic and environmental factors are known to affect the progression of the disease. This project focuses on the discovery of changes in metabolism due to the PINK1 gene mutation. PINK1 is a mitochondrially targeted, serine/threonine protein kinase PTEN-induced kinase 1 (PINK1) that protects cells from stress-induced mitochondrial dysfunction. We compare the effects of mitochondria-targeting inhibitors in patient and gene-corrected PINK1-Q456X cells, with healthy controls. Our data was generated from in vitro cultures of patient-derived human neuroepithelial stem cells, that were differentiated into dopaminergic neurons. A generic human genome-scale metabolic model was used to create a dopaminergic neuron-specific metabolic model. The steady-state solution spaces of constraint-based models of metabolism, in patient and gene-corrected PINK1-Q456X cells, were sampled and the flux distributions for each reaction were compared. Individual flux distributions may be diverse (uniform, normal, truncated, etc.), posing a challenge in statistical analysis. To address this issue, we developed an algorithm that classifies diverse flux distributions and highlights dissimilarities. Our approach allows us to study the differences in the uptake and secretion rates between the patient, gene-corrected, and healthy control models. Thereby, leading to a better understanding of the pathogenesis of PINK1-PD.

P-483 Extended Quality Control for Biocrates' Targeted Metabolomics Kits

PRESENTING AUTHOR: ***Mathias Kuhring, Max Delbrück Center (MDC) for Molecular Medicine, Germany***

CO-AUTHORS: ***Alina Eisenberger, Raphaela Fritsche, Yoann Gloaguen, Dieter Beule, Jennifer Kirwan***

Targeted mass spectrometry profiling methods optimized and validated for defined metabolites enable comprehensive routine metabolomics applications such as the analysis of larger cohorts. However, comprehensive studies require consistent processing and reliable instrumentation to minimize technical variance and interference. Consequently, multiple and reproducible controls are required to verify data quality. While standardized methods such as the Targeted Metabolomics Kits of Biocrates promise consistent and comparable measurements, they are not fully resistant to external influences. These include sample handling and processing errors, contamination, sample carryover, batch effects, intra-batch drift, edge effects, missing values of unknown origin and instrument condition. Here, we present an extensive quality control procedure for targeted data acquired using Biocrates kits designed to be complementary to the Biocrates MetIDQ software. Based on MetIDQ outputs, it combines several visualizations into a comprehensive HTML report using an R Notebook. These include, for instance, visualizations of measured and missing values, of positional irregularities with respect to acquisition sequence or well plate coordinates as well as of sample and metabolite variability and reproducibility. The tool supports Biocrates' AbsoluteIDQ® p400 HR Kit and MxP® Quant 500 Kit, however, most features apply to other Biocrates kits exportable by MetIDQ, with possible future extension to generic targeted metabolomics data. Overall, the report aids in either verifying data consistency and quality or, if necessary, in identifying pattern of interference as well as removing low quality samples or metabolites, thereby increasing confidence in data and subsequent analysis. An R package will be made available under a permissive license.

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TECHNOLOGY

P-484 Blend to avoid mixed results

PRESENTING AUTHOR: ***Daniel Malmodin, Swedish NMR Centre, Sweden***

CO-AUTHORS: ***Anders Pedersen, Göran Karlsson***

By using also blends of urine samples from participants in an NMR study the problem of variation in salt concentrations and pH, causing variation in spectrum shifts, can partly be overcome resulting in increased accuracy of assignments and precision of concentration estimates, as well as giving means to quantify each estimate in a more formal way than today.

P-485 A novel algorithm to improve NMR alignment of urine metabolomics data by spectral reordering and ridge tracing

PRESENTING AUTHOR: ***Sicong Zhang, The University of Georgia, United States***

CO-AUTHORS: ***Arthur S. Edison***

In Nuclear Magnetic Resonance (NMR) spectroscopy, the chemical shifts of some resonances are sensitive to sample properties, such as pH, salt concentration, temperature, and other matrix effects. This phenomenon challenges feature annotation and chemometric analysis in metabolomics research, leading to the necessity of data alignment processing steps. Urine is a widely used sample type in NMR metabolomics because of its simplicity in sample preparation and rich data. However, urine NMR samples are difficult to buffer and lead to large chemical shift variations. Although different alignment algorithms have been developed, none of them perfectly solve this problem. Here we present a new algorithm to improve urine NMR spectra alignment, by reordering the spectra according to the chemical shift of an internal peak then tracing responding peaks. We utilized DBSCAN-clustering to exclude mis-traced peaks, then used confidence intervals of traced peaks to find missed peaks. All traced peaks were aligned and placed at the end of the original spectra for further analysis. Two human urine datasets were used here for algorithm development. Significant improvement of peak alignment and STOCSY performance were observed using this algorithm. We will next compare results from this algorithm with other urine NMR analysis and alignment approaches. This work is expected to be generally useful for NMR processing to improve the accuracy and coverage of urine metabolomics studies.

P-486 An introduction to TameNMR (open source web-server based analysis of 1D NMR datasets)

PRESENTING AUTHOR: ***Marie Phelan, Technology Directorate, University of Liverpool, United Kingdom***

CO-AUTHORS: ***Arturas Grauslys, Andy Jones***

TameNMR is an open source web-server based toolset for analysis of 1D NMR datasets. Incorporating Bruker data and R packages, the University of Liverpool Computational Biology facility has developed a set of free-to-use tools that bridge the gap between raw NMR spectra and quantitative data analysis. Starting from raw or processed spectra, data can be normalised, aligned and scaled interactively prior to bucketing peaks and multiplets for downstream statistical analysis. The software has been developed by Dr Arturas Grauslys under the guidance of Prof Andy Jones who has a proven track record in developing server-based software solutions for MS proteomics community and it is hoped that functionality (as well as integration with other phenomenal tools) will expand with sufficient uptake by the NMR metabolomics community.

P-487 An IROA-based modified MSTUS Normalization corrects non-IROA sample-to-sample metabolite variation

PRESENTING AUTHOR: ***FELICE DE JONG, IROA TECHNOLOGIES LLC, United States***

CO-AUTHORS: ***Chris Beecher***

The IROA TruQuant (TQ) protocol uses a Long-Term Reference Standard (LTRS), a defined chemical mixture containing hundreds of metabolites, plus an Internal Standard (TQ-IS) that is chemically identical but isotopically different, to measure instrument performance and provide verifiable chemical Identification. Using these standards we have previously shown the ability to correct for the ion suppression of natural abundance experimental compounds that are paired with compounds in the TQ-IS, and we have further shown that once ion suppression is corrected, sample-to-sample normalization may be achieved using a modified MSTUS algorithm, in which unlike the original MSTUS algorithm, the experimental compounds are normalized to their Internal Standard counterparts. In this poster we provide a comparison of the original MSTUS-based normalization algorithm to this IROA-based modified-MSTUS algorithm to demonstrate that IROA-based normalization is not only significantly more accurate within a single experiment, but by normalizing to a standard mixture, will normalize not only sample-to-sample intraday, but also interday (day-to-day) analyses. In addition, we selected several compounds that were not present in either the LTRS or TQ-IS to demonstrate that the same normalization factor used to normalize compounds for which we had internal standards could be applied to compounds that did not have internal standards. While they did not normalize as accurately without the ability to correct for ion suppression, the ability to normalize was greatly improved compared with that of the original MSTUS algorithm.

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P-488 ASCA-fusion of NMR Metabolomics Data Reveals Metabolic Alternations in Response to Bioactive Milk Ingredients in Preterm Piglets with Intra-amniotic Inflammation

PRESENTING AUTHOR: ***Masoumeh Alinaghi, Department of Food Science, Aarhus University, Denmark***

CO-AUTHORS: ***Duc Ninh Nguyen, Per Torp Sangild, Hanne Christine Bertram, Johan A. Westerhuis***

Background: Analysis of multiple compartments of the same piglets can provide complementary information about the studied biological system. However, in such complicated systems, distinguishing different experimental variations by ANOVA-simultaneous component analysis (ASCA) prior to data fusion could be of importance. In this study, a dietary intervention for prenatal inflammation1 is investigated by analysis of urine and gut samples from the same piglets using the ASCA-fusion approach. Methods: Preterm pigs (n=17), subjected to intra-amniotic lipopolysaccharide (LPS, 1 mg/fetus), were administered i) standard formula, ii) bovine colostrum or iii) caseinoglycomacropeptide for five days. Collected urine and gut contents were analyzed by 1H NMR-based metabolomics. ASCA was used to separate the various sources of variation in the data related to experimental factors (time and treatment), while fusion of the ASCA-decomposed matrices was applied by using penalized exponential simultaneous component analysis (P-ESCA)2 to distinguish the common and distinct variation associated with the dietary intervention. Results: ASCA-fusion of the urine and gut metabolomes reveals the common alternation of lactate, glucose, acetate levels as well as disaccharides in response to the different dietary interventions. However, common responses related to the sex of piglets are also observed. Conclusion: ASCA-P-ESCA improves the understanding of metabolomics alternations in urine and gut content by separation of induced variations and by finding common and distinct variations in both compartments. 1 Nguyen, D.N., et al., The American journal of pathology, 188.11 (2018), 2629-2643. 2 Song, Y., et al., (2019), arXiv: 1902.06241.

P-489 AutoTuner - high fidelity, robust, and rapid data processing parameter selection tool for metabolomics data

PRESENTING AUTHOR: ***Craig Mclean, MIT, United States***

CO-AUTHORS: ***Elizabeth B Kujawinski***

Untargeted metabolomics experiments have the capability to capture an unbiased snapshot of cellular metabolism but remain challenging due to the computational complexity involved in data processing and analysis. Raw data must be processed to remove noise and to align features across samples through software tools like XCMS or MzMine2, resulting in a table of features with paired mass-to-charge (m/z) and retention-time (RT) values. This processing step requires dataset-specific parameters. Several optimization methods exist, but each design includes undesirable drawbacks. Here, we present a new method, AutoTuner, designed to optimize data processing parameters based on a novel paradigm. Instead of maximizing an optimization function like its predecessors, AutoTuner relies on statistical inferences within the distribution of raw data. We tested the accuracy and the run time of AutoTuner against the most common parameter selection tool, isotopologue parameter optimization (IPO). We also analyzed how parameter selection for AutoTuner and IPO influenced the quality of feature tables after XCMS. In our presentation, we will show that AutoTuner is a desirable alternative to existing tools, with substantially shorter computational times, easy implementation into existing metabolomics pipelines, and openly available to software developers.

P-490 NonTplus – a new R package for the high throughput processing of high resolution mass spectrometry data

PRESENTING AUTHOR: ***Tobias Schulze, Helmholtz Centre for Environmental Research - UFZ, Germany***

CO-AUTHORS: ***Erik Müller, Caroline Huber, Marc Stöhr, Werner Brack, Martin Krauss***

High resolution mass spectrometry is a key analytical technology in the identification of targets, suspects and unknowns for the profiling of environmental or human samples. In larger scale environmental surveys or human cohort studies, hundreds or thousands of samples could emerge. Assuming that these samples are measured at least once in positive and negative mode, a large number of raw mass spectral files is derived. NonTplus is a new pipeline which automatizes the whole processing including peak picking, gap filling, blank peak elimination, peak alignment, annotation, quantification and the export of peak lists for post-analysis. NonTplus implements a new gap filling algorithm which improves missing value imputation with less false positives compared to existing algorithms. The pipeline is tailored to run on a HPC cluster and a future implementation in Galaxy is planned.

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TECHNOLOGY

P-491 On the Interpretability of O-PLS Filtered Models

PRESENTING AUTHOR: ***Barry M. Wise, Eigenvector Research, Inc., United States***

CO-AUTHORS: ***Federico Marini, Frank Westad***

Orthogonal PLS, introduced originally by Trygg and Wold in 2002 [1], is a patented algorithm that has received much attention for its perceived ability to simplify and thus improve regression and classification model interpretation. Since its introduction, it has been shown by Ergon [2] and Kemsley and Tapp [3] that results identical to the original O-PLS formulation can be obtained by post-processing conventional PLS models in a non-patented way. This demonstrated, unequivocally, that O-PLS models have predictive properties identical to their non-rotated versions. The authors did not, however, consider the interpretability of the models at length. In this poster we explore the issue of interpretability of O-PLS models by applying the method to carefully constructed simple systems and well characterized data. It is demonstrated that O-PLS results match the true underlying (first principle) components under only very specific conditions which are typically not met. O-PLS results are shown to be a strong function of the correlation between the factor of interest and interfering components even under mild conditions, (small correlations). In simulated binary expression data it is shown that O-PLS is actually more sensitive to chance correlation than the conventional PLS regression vector. [1] J. Trygg and S. Wold, "Orthogonal Projections to Latent Structures (O-PLS)," J. Chemo, 16, 119-128, 2002. [2] R. Ergon, "PLS post-processing by similarity transformation (PLS+ST): a simple alternative to OPLS," J. Chemo, 19, 1-4, 2005. [3] E.K. Kemsley and H.S. Tapp, "OPLS filtered data can be obtained directly from non-orthogonalized PLS1," J. Chemo, 23, 263-264, 2009

P-492 An Improved Lipid Profiling Workflow Demonstrates Disrupted Lipogenesis Induced with Drug Treatment in Leukemia Cells

PRESENTING AUTHOR: ***MARK SARTAIN, Agilent Technologies, United States***

CO-AUTHORS: ***Genevieve Van de Bittner, Xiangdong Li, Jeremy Koelmel, Adithya Murali, Sarah Stow***

While shotgun lipidomics has advanced the field of lipid analysis, it suffers from limitations including the failure to distinguish isobaric species which may be of biological importance. This has led to a shift in the field of lipidomics to chromatographic-based lipid profiling approaches using high performance liquid chromatography coupled to high resolution mass spectrometry. Confident lipid annotation requires data acquisition at the MS/MS level to enable product-ion spectral matching against in silico generated databases. In this study, a novel software tool was employed which uses Bayesian scoring to assign lipid class annotation and a non-negative least squares fit with a theoretical lipid library (LipidBlast) to annotate the iterative mode MS/MS spectra. The tool takes special care not to overannotate lipid entities by only providing the level of structural information confidently informed by the MS/MS spectra. The tool quickly generates an accurate mass-retention time database in an automated fashion, and the resulting database annotates MS1 lipid profiling data. We applied this novel workflow to study lipidome alterations of the acute-myeloid-leukemia K562 cell line in response to a combination of the drug candidates bezafibrate (BEZ) and medroxyprogesterone acetate (MPA). The resulting analysis revealed several cellular changes in response to drug treatment, including a decrease in diacylglycerols, an increase in triacylglycerols, and differences in fatty acyl components. Utilization of the new lipid analysis workflow provided a more comprehensive lipid annotation than can be achieved by traditional approaches, and the results supported that BEZ/MPA may exert anticancer properties through disruption of lipogenesis.

P-493 Developing a systematized and integrated workflow for large-scale LC-MS data processing and cross-study investigations

PRESENTING AUTHOR: ***Sajjan Mehta, University of California, Davis, United States***

CO-AUTHORS: ***Gert Wohlgemuth, Diego Pedrosa, Sili Fan, Oliver Fiehn***

We present on the development of an enterprise-grade LC-MS data processing pipeline, LC-BinBase, and its integration with existing software to serve as an automated data management strategy for metabolomics and lipidomics studies. LC-BinBase implements MS-DIAL concepts and techniques to perform standardized data processing and feature annotation in a highly scalable capacity by utilizing cloud-based AWS Lambda services and Fargate clusters. Sample and study management is handled by the in-house MiniX and Stasis services and defines all sample preparations and quality controls. Acquired data are pre-processed and converted on the fly to the open mzML data format and are stored on AWS S3 for processing long-term storage. The converted samples are automatically scheduled for data processing by LC-BinBase using the study definition, instrument type and matrix information to tune the processing parameters. Feature identification uses m/z-RT libraries and MS/MS mass spectral libraries that are method-specific to minimize erroneous annotations. Identified features are stored in a dedicated instance of MassBank of North America (MoNA), and later consensus spectra are uploaded to the public MoNA database as metabolites are identified and manually confirmed. Each sample's validated annotations along with its matrix information are aggregated by BinVestigate to provide unique insight into the prevalence of individual metabolites within specific species and organs or components from across all historical studies. In addition, the use of standardized quality controls and retention time normalization enables cross-study investigation utilizing Systematic Error Removal using Random Forest (SERRF) for robust sample normalization.

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TECHNOLOGY

P-494 BioMagResBank: Database and Tools for NMR Metabolomics Analysis

PRESENTING AUTHOR: *Pedro Romero, University of Wisconsin Madison, United States*

CO-AUTHORS: *Hamid R. Eghbalnia, Hesam Dashti, Naohiro Kobayashi, Jonathan R. Wedell, Kumaran Baskaran, Takeshi Iwata, Masashi Yokochi, Dimitri Maziuk, Hongyang Yao, Toshimichi Fujiwara, Genji Kurusu, Eldon L. Ulrich, Jeffrey C. Hoch, John L. Markley*

The Biological Magnetic Resonance Data Bank (BioMagResBank or BMRB), founded in 1988, serves as the archive for data generated by Nuclear Magnetic Resonance (NMR) spectroscopy of biological systems. NMR spectroscopy is unique among biophysical approaches in its ability to provide a broad range of atomic and higher-level information relevant to the structural, dynamic, and chemical properties of biological macromolecules, as well as reporting on metabolite and natural product concentrations in complex mixtures and their chemical structures. BMRB stores experimental and derived data from biomolecular NMR studies on both biopolymers and bioactive small compounds. BMRB supports metabolomics NMR studies through a library of a variety of 1D and 2D NMR spectra of pure compounds (including metabolites, natural products, drugs, and compounds used for screening in drug discovery) and through its adoption of novel analytic tools, like the ALATIS unique atom identifiers, which are universal and based solely on the 3D structure of the compound and the InChI convention, and GISSMO spin matrices, which enable accurate simulation of compound and mixture spectra at any field strength. The combination of unique ALATIS naming and parameterized spectra offers the users of BMRB data a distinctive benefit in terms of robustness and reproducibility, as embodied in the FAIR principles for data resources, which are that data should be Findable, Accessible, Interoperable, and Reusable. Supported by NIH Grants NIH Grants R01GM 109046, P41GM103399, and P41GM111135R01.

P-495 Estimating Partial Correlation Networks Leveraging Prior Information with Applications to Metabolomics Data

PRESENTING AUTHOR: *George Michailidis, University of Florida, United States*

CO-AUTHORS: *Jiahe Lin, Alla Karnovsky, Gayatri Iyer, William Duren*

The problem of estimating networks from high-dimensional Omics data has received a lot of attention due to their usefulness in providing insights into interactions amongst biomolecules under different diseases or experimental conditions. Partial correlation networks provide a useful technical framework for the task at hand. However, the limited number of samples available in many studies leads to estimation of very sparse (and fragmented) networks that makes them hard to interpret. To that end, we propose an estimation framework that leverages external information provided in the form of similarities across network edges. We formulate a regularized pseudo-likelihood framework, develop a fast distributed proximal gradient descent algorithm to compute the network structure and discuss selection of tuning parameters. We illustrate the proposed framework and the resulting algorithm by reconstructing and comparing the networks obtained from metabolomic and lipodomic profiling of three groups of samples, one suffering from Crohn's disease, another from ulcerative colitis and the third of normal controls. We further tested for enrichment modules extracted from the estimated networks that identified important alterations in both network structure and expression levels of interacting metabolites/lipids across the three groups of interest.

P-496 Updates to xcms: simplified raw data access and enhanced MS level > 1 capabilities

PRESENTING AUTHOR: *Johannes Rainer, Institute for Biomedicine, Eurac Research, Italy*

CO-AUTHORS: *Laurent Gatto, Steffen Neumann*

The xcms Bioconductor package is one of the standard toolboxes for the preprocessing of untargeted metabolomics data. Here we present recent updates to xcms, which re-use and build upon the support for memory-efficient parallel processing capabilities in the MSnbase Bioconductor software package for proteomics and general mass spectrometry data handling. We have improved large-scale experiment data analysis through memory-efficient parallel processing capabilities and simplify raw spectra data access throughout the whole preprocessing task. This comprises also dedicated functionality to extract ion chromatograms/traces from the original files and to perform chromatographic peak detection directly on such chromatographic data. Besides paving the road for MRM/SRM data analysis with xcms, it also allows to evaluate different peak detection settings on selected signals before applying them to the whole data set. Along these lines, we also implemented new visualization capabilities aiding in the definition and evaluation of data set-specific settings for the various preprocessing algorithms. Finally, import of MRM/SRM raw data has been added and a framework for the identification of MS2 spectra for identified chromatographic peaks was implemented.

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*AWARD WINNERS

TECHNOLOGY

P-497 Prediction, quantification and correction of impaired plasma sample quality induced by pre-analytical errors using LC-MS untargeted metabolomics

PRESENTING AUTHOR: ***Rui Zheng, Uppsala University, Sweden***

CO-AUTHORS: ***Lin Shi, Rikard Landberg, Huma Zafar, Åsa Torinsson Naluai, Carl Brunius***

The quality of biobanked blood samples is of great importance for reliable and accurate determination of metabolites. Pre-analytical handling is one of the most important factors for sample quality. We used untargeted LC-MS metabolomics to evaluate the influence of pre-analytical management on 471 plasma samples from 28 individuals. Random forest modelling accurately predicted pre-centrifuge temperature (classification rate 81%) and time (Q2 = 0.82) as key factors for pre-analytical sample quality. Fasting status, however, did not affect the metabolome reproducibly among individuals. Thirteen and eight metabolites were selected in metabolite panels for highly accurate prediction of temperature and time, respectively. Several metabolites responding to temperature/time interaction in linear regression showed significant difference from 30 to 120 min at 25 or 37 °C compared to 4 °C, whereas temperature could not be accurately predicted at <30 min. Moreover, the changes in plasma metabolome were modelled per cluster at each temperature and pronounced at 4 °C because intensity of lipids-like and organic acids features dramatically declined from 0 until 100 min. Furthermore, only minor to moderate (0 to 25%) correction of data quality could be achieved by normalizing feature data to 0 min based on metadata on time, indicating that the induced variability to a large degree is non-systematic. We conclude that the metabolite profile changes rapidly with pre-centrifugation delay times even at 4 °C. Handling of blood samples from needle to freezer should be completed as soon as possible, preferably at 25 °C with pre-centrifugation delays less than 30 min.

P-498 MetaboAnalystR 2.0: From Raw Spectra to Biological Insights

PRESENTING AUTHOR: ***Jasmine Chong, McGill University, Canada***

CO-AUTHORS: ***Mai Yamamoto, Jianguo Xia***

Global metabolomics based on high-resolution liquid chromatography mass spectrometry (LC-MS) has been increasingly employed in recent large-scale multi-omics studies. Processing and interpreting these complex datasets have become a key challenge in current computational metabolomics. We therefore present MetaboAnalystR 2.0, an R package to support end-to-end LC-MS based global metabolomics data analysis from spectral processing to biological insights. Compared to its predecessor, this new release integrates XCMS and CAMERA to support raw spectral processing and peak annotation. It also features high-performance implementations of Mummichog and GSEA algorithms to predict pathway activities directly from MS peaks. The application and utility of the MetaboAnalystR 2.0 workflow are demonstrated using a clinical dataset of pediatric inflammatory bowel disease (IBD). Functional analysis identified perturbations in Bile acid biosynthesis and Vitamin D3 metabolism, both of which are well-known mechanisms in IBD. This highlights the ease of which MetaboAnalystR 2.0 can be used to gain biological insights and generate hypotheses for future experimental validation. In summary, MetaboAnalystR 2.0 offers a unified and flexible workflow that enables end-to-end analysis of LC-MS metabolomics data within the open-source R environment. The R package is freely available from the GitHub repository (https://github.com/xia-lab/MetaboAnalystR).

P-499 Galaxy on Site: A flexible and reliable path to processing metabolomics data reproducibly and collaboratively

PRESENTING AUTHOR: ***Arthur Eschenlauer, University of Minnesota - Twin Cities, United States***

CO-AUTHORS: ***Mark Esler, Timothy Griffin, Adrian Hegeman***

Untargeted metabolomics LC-MS experiments can generate large numbers of large files; before the results can be interpreted, it is necessary to perform many preprocessing, annotation, and statistical analysis steps, each of which may have its own particular parameters. Galaxy provides a web-based interface to capture these parameters into reproducible, reusable, shareable workflows. Getting started can be as simple as using one of the established public Galaxy instances, engaging high-performance computing resources, or running Galaxy on a workstation. A research group may have unique needs in the areas of access, collaboration, or simplified transfer and life-cycle management of sizeable datasets; new, in-house authored tools may also need to be incorporated into Galaxy workflows. A research-group specific Galaxy “appliance” may practically address these needs; however, this requires a sustainable way to administrate a small-scale, high-availability system. We have been running such a system for several years and are encapsulating these functionalities into an “appliance” that can be implemented in a broad spectrum of laboratory settings. Our initial efforts have been focused on scaling the system appropriately, system backup and recovery, balancing cost of storage against available size, and integrating the system with instrument workstations on a laboratory intranet. From the outset, the system was designed to balance security with usability. We have applied this solution to our plant metabolomics research and found that, with minimal instruction, users can work independently and provide feedback on usability and functionality issues in prepublication versions of Galaxy tools.

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TECHNOLOGY

P-500 Which step is the most crucial in sample preparation procedure for GC-MS metabolomics? Design of Experiments approach.

PRESENTING AUTHOR: ***Julia Jacyna, Dept. of Biopharmacetics and Pharmacodynamics, Medical University of Gdansk, Poland***

CO-AUTHORS: ***Marta Kordalewska, Joanna Raczak-Gutknecht, Marta Stawiszyńska, Michał Jan Markuszewski***

Design of Experiments (DoE) consists of making specific and controlled modifications in a studied system in order to create a mathematical model that will allow to predict how monitored responses are affected by applied modifications. In other words, the use of DoE approach allows to screen the most important factors, predict relationships between them and generate the most optimal settings in order to get the most favorable response while saving the time spent on the optimization and reducing the cost of analysis. Its main advantage is the ability to provide optimal parameters' settings by performing a minimal number of experiments. The objective of the study was to develop and optimize a simple method for preparation of human urine samples for determination of concentration of previously selected metabolites by means of GC-MS/MS analysis. A rapid, simple and reliable method is necessary for targeted metabolomics analysis. Moreover, since sample preparation step for GC-MS/MS is usually very complicated, time-consuming and requires the use of toxic reagents, implementation of Design of Experiments was reasonable. Fractional Factorial Design was implemented as a screening procedure in order to evaluate the significance of variables. The most crucial steps of urine sample preparation procedure were identified with the use of two-step plan, based on the evaluation of more than a dozen of parameters with limited number of experiments. Firstly, time- and temperature-dependent factors were evaluated and subsequently, concentration and volume of reagents used were taken into account.

P-501* Analytic correlation filtration: A new tool to reduce analytical complexity of metabolomic datasets

PRESENTING AUTHOR: ***Stephanie Monnerie, Université Clermont Auvergne, INRA, UNH, Mapping, France***

CO-AUTHORS: ***Melanie Petera, Bernard Lyan, Pierrette Gaudreau, Blandine Comte, Estelle Pujos-Guillot***

Metabolomics generates complex data that need dedicated workflows to extract the meaningful information. For biological interpretation, experts are mainly focusing on metabolites rather than on the redundant different analytical species. Moreover, the high degree of correlation in datasets is a constraint for the use of statistical methods. In this context, we developed a new tool to detect analytical correlation into datasets. The algorithm principle is to group features from the same analyte and to propose one single representative per group. The user can define grouping criteria with various options including correlation coefficient, retention time, mass defect information. The representative feature can be determined following four methods according to the analytical technology. The present tool was compared to one of the most commonly used free package proposing a grouping method: 'CAMERA', using its Galaxy version 'CAMERA.annotate' available in Workflow4Metabolomics (W4M; http://workflow4metabolomics.org). To illustrate its functionalities, a published dataset available on W4M (Thevenot et al., 2015) was used as an example. Within the 3,120 ions of the dataset, the tool allowed creating 2,651 groups, meaning that 15% of ions are proposed to be filtered because of analytical redundancies. The proposed tool subdivided more than 20 groups of more than 10 ions into smaller ones corresponding to individual annotated metabolites, thus demonstrating the efficiency and relevance of the present approach. As a key element in metabolomics data analysis, the tool will be available via the web-based galaxy platform W4M with different output files for network vizualisation and for further data analysis within workflows.

P-502 Comets-Analytics: a centralized computational framework for consortia level meta-analyses

PRESENTING AUTHOR: ***Ewy Mathe, Ohio State University, United States***

CO-AUTHORS: ***Moore, Krista Zanetti, Kai-Ling Chen, Dave Ruggieri, Ella Temprosa***

Metabolomics is increasingly applied in large-scale epidemiological studies to uncover metabolites associated with physiological states (e.g. age, disease). The National Cancer Institute-led "Consortium of Metabolomics Studies" (COMETS) includes > 45 international prospective cohorts with serum metabolomics profiles and detailed phenotypic data. To support meta-analysis of these studies at a consortia level, we created a centralized computational infrastructure, Comets-Analytics. Comets-Analytics was built with the following guiding principles in mind: 1) minimal burden on analyst time, 2) reproducibility, 3) data privacy, 4) adherence with FAIR guidelines, 5) usability. The software supports harmonization of metabolite names across different platforms used in COMETS and implements partial correlation modeling. Notably, Comets-Analytics includes "smart analytics", which internally performs extensive data and model checks to ensure that models are valid, and that the model building process is reproducible. Meaningful warnings (e.g. adjustment variable is dropped because it only has one unique value) and errors (e.g. adjustment and stratification variables are the same) are returned to precisely inform users about how to fix their input data and about what models are run. Further, the web application includes interactive tables and plots to empower users to promptly and globally assess results. With Comets-Analytics, cohorts analyze their own data using a common data format and standardized results are sent centrally for meta-analysis. This streamlined approach greatly facilitates large consortia studies and helps ensure integrity, and reproducibility of results. Comets-Analytics, including detailed documentation, is available as an R package at https://github.com/CBIIT/R-cometsAnalytics, and can be run directly from our servers at http://comets-analytics.org/.

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TECHNOLOGY

P-503 Composite Score: A Multivariate Correlation for Comparison of Metabolomics-Based Studies of Complex Mixtures

PRESENTING AUTHOR: *Joshua Kellogg, University of North Carolina at Greensboro, United States*

CO-AUTHORS: *Olav M. Kvalheim, Nadja B. Cech*

Untargeted metabolomics analyses, where an entire measurable metabolome is analyzed without defined biomarker compounds to guide analysis, is useful when the study has no a priori chemical or mechanistic hypotheses. However, successful untargeted metabolomics studies rely on effective statistical analysis of the metabolomic dataset to guide interpretation and inform conclusions. A primary statistical tool, principal component analysis (PCA), results in the selection of maximum three components from a larger model to visually represent similarity within the entire dataset. This limits the comprehensiveness of the model and can yield poor discrimination between samples. Other metrics of similarity also have limitations when considering untargeted metabolomics studies. Here we have developed a new statistical metric, the composite score (CS), as a univariate statistic that incorporates multiple principal components to enable quantitative comparisons among metabolomics datasets. By integrating the scores and loadings components of the significant components from the original PCA model, the CS provides an advantageous measure of similarity, enabling more quantitative comparisons than are possible with visual inspection of a PCA scores plot or hierarchical cluster analysis (HCA). Several case studies focusing on complex natural product mixtures – green tea (Camellia sinensis) and goldenseal (Hydrastis canadensis) dietary supplements – highlight the utility of composite scores to evaluate similarity and identify outliers within a sample set.

P-504 Integrating 4D peak picking of LC-TIMS-MS/MS data into GNPS feature based molecular networking for metabolomics and lipidomics analysis

PRESENTING AUTHOR: *Florian Zubeil, Bruker Daltonik GmbH, Germany*

CO-AUTHORS: *Nikolas Kessler, Heiko Neuweger, Sven Meyer, Ulrike Schweiger-Hufnagel, Aiko Barsch*

As throughput of metabolomic and lipidomic analyses continuously expands, effective workflows for analyzing the resulting datasets are of increasing importance. Molecular networking in recent years has become a vital tool in the metabolomics community as it quickly allows the identification of compounds with similar fragmentation patterns which are often structurally related. This also allows propagation of annotations from known compounds to related derivatives. While this approach mainly focusses on the fragment spectra, important information can be deduced from the precursor spectra, i.e. intensity, accurate mass and isotopic pattern of the analytes. Herein, we present a workflow to integrate analyte information for untargeted profiling from the software MetaboScape into GNPS feature based molecular networking. The nodes in the resulting molecular network are enriched by useful information about the precursor ions like the intensity in individual samples, molecular formula, annotation, CCS values, group mean and maximum intensity. The latter information are important indicators to assess distribution of a specific analyte between sample groups (by group mean). Likewise, interpretation of the resulting molecular network is greatly simplified by displaying generated molecular formulas instead of precursor masses as node labels. Additionally, this workflow enables the integration of 4D peak picking results from PASEF-MS/MS data into GNPS molecular networking which is demonstrated on a lipid sample. This enables a straight forward processing of TIMS-MS data including the benefit of cleaner MS/MS spectra of co-eluting analytes for molecular networking which are generated by ion mobility separation.

P-505 Hierarchical Bayesian models for Stable Isotope Resolved Metabolomics: a unified framework for testing hypotheses about total abundance and isotopologue distribution

PRESENTING AUTHOR: *Patrick J. Trainor, University of Louisville, United States*

CO-AUTHORS: *Pawel K. Lorkiewicz, Joshua K. Salabei, Bradford G. Hill*

The analysis of data from Stable Isotope Resolved Metabolomics (SIRM) experiments presents a unique statistical challenge. First, experimental manipulations (e.g. gene knockout or treatment with an enzymatic inhibitor) may change between phenotypes: the concentration or pool of a metabolite present, the fractional distribution of the metabolite pool within isotopologues, or both. Second, multiplicity concerns arise from conducting univariate statistical tests at the isotopologue level between phenotypes. We propose a hierarchical Bayesian model for testing hypotheses in SIRM experiments that is well-suited for addressing these challenges. This model assumes Gaussian prior distributions for the total abundance of a metabolite, Dirichlet prior distributions for the fractional distribution of abundances within isotopologues, and Gaussian priors to account for within-phenotype variability as well as measurement error. Gibbs sampling, a Markov chain Monte Carlo technique, is utilized to simulate the joint posterior distribution of model parameters given observed experimental data. From the joint posterior distribution hypotheses regarding both total metabolite abundance and isotopologue distribution can be tested. We demonstrate an application of the methodology to a 15N2, 13C5-glutamine labeling experiment conducted in order to evaluate the effect of aminoooxyacetic acid (an inhibitor of aminotransferases including aspartate aminotransferase) treatment (AOA) in murine cardiac mesenchymal cells isolated on the basis of c-kit positivity. Metabolites extracted from AOA+ and AOA- treated cells were detected by FTICR-MS and relative abundances were quantified. We report differences in isotopologue distributions between AOA+ and AOA- cells in malate and glutamate; and differences in total abundances of malate, aspartate, and glutamate.

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TECHNOLOGY

P-506 Metabolomic profiling identifies a systemic suppression of steroid metabolism among prevalent asthma cases; are inhaled steroids the cause?

PRESENTING AUTHOR: *Priyadarshini Kachroo, Brigham & Women's Hospital, United States*

CO-AUTHORS: *Priyadarshini Kachroo, Rachel S. Kelly, Mengna Huang, Isobel Stewart, Claudia Langenberg, Scott T. Weiss, Jessica A. Lasky-Su*

Asthma is a complex disease, often effectively treated with the use of inhaled corticosteroids (ICSs). We aimed to identify metabolomic signatures of asthma and evaluate the impact that ICSs may have on the overall plasma metabolomic profile. The current study included 10,754 participants from the population-based EPIC-Norfolk cohort with data available for prevalent physician-diagnosed asthma. Metabolomic profiling of plasma was conducted using ultrahigh-performance liquid chromatography and tandem mass spectrometry. We assessed individual metabolite associations with asthma using multivariable logistic regression models and evaluated the impact of ICS use on those findings. We replicated these findings using data from 613 individuals in the Partners Biobank (PB). After quality control, we identified 858 known metabolites, of which 27 (3.1%) were associated with prevalent asthma after Bonferroni multiple-comparison correction ($P<5.8e-5$). The top two associations: dehydroisoandrosterone sulfate (DHEA-S) and cortisone were decreased in asthmatics (OR=0.65,P=1.4e-27; OR=0.72,P=7.8e-20 respectively). 15/27 metabolites replicated in Partners Biobank ($P<0.05$), including strong associations between the top two EPIC findings and asthma: DHEA-S and cortisone (OR=0.36,P=2.7e-4; OR=0.30,P=3.0e-5 respectively). Notably, all 15 metabolites were in the corticosteroid, pregnenolone, and androgenic steroid pathways and were markedly reduced in asthmatics (ORs=0.65-0.81). Further investigation demonstrated that these metabolites had consistent negative associations with ICS use. These findings suggest that significant suppression of multiple steroid pathways in asthmatics could be modulated by ICSs, which merits from further investigation. The consistent negative relationship between ICS use and steroid metabolites further suggests that ICSs may have stronger systemic effects on circulating plasma steroid levels than is currently recognized.

P-507 Hierarchical preprocessing for LC/MS metabolomics data generated in multiple batches

PRESENTING AUTHOR: *Tianwei Yu, Emory University, United States*

CO-AUTHORS: *Qin Liu, Douglas Walker, Karan Uppal, Shuzhao Li, ViLinh Tran, Dean P. Jones*

With the growth of metabolomics research, more and more studies are conducted on large numbers of samples. Due to technical limitations of the Liquid Chromatography – Mass Spectrometry (LC/MS) platform, the samples need to be processed in multiple batches. Across different batches, we often observe differences in data characteristics. In this work, we specifically focus on data generated in multiple batches on the same LC/MS machinery. Traditional preprocessing methods treat all samples as a single group, which makes it necessary to use larger m/z and retention time tolerance levels in order to allow for between-batch differences. Such an approach is sub-optimal, as it can result in errors in the alignment of peaks, which cannot be corrected by batch effect correction methods applied after preprocessing. To address this issue, we developed a new approach that process the data in a hierarchical manner – first within batch and then between batch. Different parameter settings can be adaptively found for within-batch and between-batch quantification and alignments. The method is implemented in the existing workflow of the apLCMS platform. Analyzing data with multiple batches, both generated from standardized plasma samples and from real biological studies, the new method resulted in feature matrices with higher consistency. The method can be useful for large studies involving multiple batches.

P-508 Integrated workflow with quality control for large cohort and clinical metabolomics research using robust hardware and signal correction

PRESENTING AUTHOR: *Nikolas Kessler, Bruker, Germany*

CO-AUTHORS: *Sebastian Goetz, Ulrike Schweiger-Hufnagel, Matthias Szesny, Aiko Barsch, Sven W. Meyer, Matthew R. Lewis, Nikolas Kessler*

Metabolomics research relies on precision measurement of statistically powered sets of hundreds or thousands of samples. First, this requires robust analytical hardware with long term stability, capable of generating high precision data. Second, processing of large datasets may require additional mathematical correction to compensate for systematic changes in observed signals as samples interact with the analytical system affecting its performance. We investigated the long-term stability of an LC-HR-QTOF system by measuring a batch of more than 1000 urine samples and monitoring the effect of data acquisition on MS ion source contamination and detector aging. To address the remaining within-batch intensity drifts we present new software: First, a fully automated software workflow allows for the automated correction of intensity drifts, improving data precision and statistical reliability. Second, an interactive and intuitive visualization provides rapid feature-wise review of intensity drifts (and their corrections) as well as detection of statistical outliers. Run-order signal drift correction effectively reduced the relative standard deviation (RSD) of feature intensities within sample groups measured across replicate quality control sample measurements. This also increased the number of analytes which meet the requirements of an RSD below 20%, a typical cut-off. Visually this improvement was also observed in PCA, with more closely clustering of sample groups. In summary, we present a workflow for population and clinical metabolomics research enabled by robust LC-HRMS hardware and software allowing filtering and correction for signal drift effects.

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TECHNOLOGY

P-509 Detect and Quantify Sources of Variability in Metabolite Measurement in a Japanese population

PRESENTING AUTHOR: *Ayano Takeuchi, Keio University, Japan*

CO-AUTHORS: *Sei Harada, Taichi Shimazu, Taiki Yamaji, Norie Sawada, Junko Ishihara, Ribeka Takachi, Kazutoshi Nakamura, Junta Tanaka, Manami Inoue, Motoki Iwasaki, Hiroyasu Iso, Masahiro Sugimoto, Akiyoshi Hirayama, Tomoyoshi Soga, Masaru Tomita, Shoichiro Tsugane, Toru Takebayashi*

Our study purpose is to quantify sources of variability in urine and plasma metabolite concentrations measured using capillary electrophoresis-mass spectrometry and detect sources of variability in metabolite measurements. We measured metabolite concentrations of using the samples from Japan Public Health Center-Based Prospective Study for the Next Generation (JPHC-NEXT) validation study in Japan (J Epidemiol. 2016;26:420-32.). We used twenty-four-hour urine collections and plasma specimens of 253 men and women aged 40–74 years from five areas in the study, collected at 2 time points, baseline (2012) and same period of next year (2013) . We randomly selected 43 samples from study subjects and dispense specimens into 3. We measured some 3 dispensing specimens sequentially to detect the sum of the squared deviations of 'pure measurement error'. We layout and measured some 3 dispensing specimens in different position of the same batch to detect 'within batch variation of measurement'. We layout and measured remained 3 dispensing specimens in same positon of different batch to detect 'between batch variation of measurement'. We will show the proportion of these 3 types of variation (pure measurement error, within batch variation, between batch variations) and variation between times (2012 to 2013) for all metabolite (123 metabolites for urine, 102 metabolites for plasma) we measured on our poster.

P-510 Virtual Metabolomics Mass Spectrometer (ViMMS): A Mass Spectrometry Simulator for Comparing Different Fragmentation Strategies in Metabolomics

PRESENTING AUTHOR: *Joe Wandy, Glasgow Polyomics, United Kingdom*

CO-AUTHORS: *Vinny Davies, Justin J.J. van der Hooft, Ronan Daly, Simon Rogers*

Liquid-Chromatography (LC) coupled to Tandem mass spectrometry (MS/MS) is widely used in identifying small molecules in untargeted metabolomics. However, the development of new MS/MS acquisition strategies is hampered by the lack of simulators that let researchers prototype and compare different fragmentation strategies before validations on real machines. Although some simulators exist, they are typically focused on proteomics and do not include simulation of MS2 acquisition within a chromatographic run. We introduce Virtual Metabolomics Mass Spectrometer (ViMMS), a modular metabolomics LC-MS/MS simulator framework that allows for real-time scan-level control of the MS2 acquisition process in-silico. ViMMS can generate new data based on kernel density estimates trained on empirical data, or generate data that resembles real data from a list of user-defined chemical formulas. Alternatively, pre-existing data can be re-run in-silico with different fragmentation strategies. Samples can be exported as .mzML files, and different fragmentation controllers compared. ViMMS is also extendable with additional spectra generation processes and noise models. We will show results from experiments that compare different fragmentation strategies. First ViMMS will be used to take the output of a real LC/MS analysis and examine the effect of varying N in Top-N Data Dependent Acquisition protocol. We will also demonstrate how ViMMS can be used to compare published acquisition strategies, e.g. Data-set-Dependent Acquisition (DsDA) and Nested Data-Independent Acquisition (DIA). We expect that ViMMS will save development time by allowing for offline evaluation of novel fragmentation strategies and optimisation of fragmentation strategy for a particular sample.

P-511 Open source software platform for mass spectrometry based non-target screening in the environment

PRESENTING AUTHOR: *Rick Helmus, Institute for Biodiversity and Ecosystem Dynamics, Netherlands*

CO-AUTHORS: *Vittorio Albergamo, Olaf Brock, John Parsons, Pim de Voogt*

Chemical analysis has been widely applied over the past decades to characterize both natural and anthropogenic compounds within our environment. A 'non-target' approach becomes increasingly adopted where hundreds to thousands of unknown chemicals are screened simultaneously. At this scale, tools are crucial to automatize extraction, prioritization and identification of chemicals of potential interest. The various software tools now available typically solve only part of the workflow and may lack functionality specifically required for environmental sciences. As a consequence, the need to combine tools may require familiarizing with various software environments, tedious transformation of in-between datasets and complicating reproducible non-target research. We are developing an R based open source software platform which provides a common interface for non-target analysis tailored for environmental sciences. Existing software solutions (e.g. XCMS, OpenMS, CAMERA, and MetFrag) are utilized to provide a typical non-target workflow such as extraction of features, calculation of chemical formulae and tentative compound identification. A common interface to these tools allows easy incorporation of tested algorithms and comparison of their output. Other functionalities include filtering and prioritization of data, interactive and static reporting and interoperability with vendor software. Our software is currently being applied in various non-target studies conducted in our institute. Examples include the chemical characterization of dissolved organic matter to study its stabilization in podzols, the identification of small and polar emerging polar contaminants to study their removal by reverse osmosis during water treatment and elucidation of transformation products of biocides released in constructed wetlands.

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*AWARD WINNERS

TECHNOLOGY

P-512 A new method for enhancing gap filling accuracy and specificity in high resolution mass spectrometry data processing

PRESENTING AUTHOR: ***Erik Mueller, Helmholtz Centre for Environmental Research - UFZ, Germany***

CO-AUTHORS: ***Werner Brack, Martin Krauss, Tobias Schulze***

Liquid chromatography high resolution mass spectrometry is a key analytical technology in the identification of targets, suspects and unknowns for profiling in environmental screening and metabolomics. By default, peak picking uses heuristic algorithms for the extraction of individual ion chromatograms (EICs) from chromatographic raw data and identification of peaks in these EICs. But heuristic methods cannot account for all types and shapes of noise occurring in the data. While some algorithms exist to mitigate this problem, most gap filling approaches result in many false positives and negatives. We present here a novel gap filling method that utilizes the similarity between the extracted ion chromatograms of a peak in different samples to deduce where previously undetected peaks can be found while also keeping the number of inaccurately categorized peaks at a minimum. With our new approach, the accuracy of categorized peaks was increased by 51% compared to the peak finder in MZmine.

P-513 A full workflow for machine learning techniques in integrative multiomics studies as part of the COVAIN toolbox

PRESENTING AUTHOR: ***Xiaoliang Sun, University of Vienna, Austria***

CO-AUTHORS: ***Wolfram Weckwerth***

The Vienna Metabolomics Center has established open-source and cross-platform workflows for computational mass spectrometry, integrative multi-omics analysis and predictive modelling for clinical, biochemical, agricultural and ecological studies. All algorithms are implemented in the toolbox COVAIN (1). High resolution mass spectral raw data are processed with an algorithm called mzFun which identifies and aligns thousands of compounds over hundreds of samples from MS/MSn fragmentations, molecular formula, isotopomer pattern and internal and external MS libraries. From this initial annotation biochemical pathways are assigned to unknown m/z features with an algorithm called mzGoupAnalyzer (2). Processed metabolomics, proteomics, transcriptomics, phenotypical and physiological data are imported into COVAIN, which provides rigorous statistical tools for data mining from data cleaning, imputation, uni- and multi-variate statistics including ANOVA, PCA, ICA, PLS, correlation, clustering, Granger causality, multiple regression up to advanced machine learning procedures. These algorithms include multivariate best subset selection by genetic algorithm, classifiers like SVM, DA, KNN and ensemble methods as well as ROC/AUC diagnostics. Further, statistical network inference, visualization, modularity analysis, KEGG pathway enrichment analysis are implemented. COVAIN is also featured with an experimentally validated inverse Jacobian calculation that infers biochemical regulation Jacobian matrix directly from genome-scale metabolomics covariance data (1,3). During this processes high quality editable figures are provided. All these computational mass spectrometry and data mining tools are organized in an All-In-One tool with graphical user interface. 1. Sun X & Weckwerth W (2012) Metabolomics 8(1):81-93. 2. Doerfler et al. (2014) Plos One 9(5):e96188. 3. Doerfler et al. (2013) Metabolomics 9(3): 564-574.

P-514 UMetDIA: Advancing SWATH-MS Based Untargeted Metabolomics

PRESENTING AUTHOR: ***Ruohong Wang, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, China***

CO-AUTHORS: ***Yandong Yin, Zheng-Jiang Zhu***

Advancing SWATH-MS Based Untargeted Metabolomics through High-Coverage Spectral Library Building and Quantitative Ion Selection. Data-independent acquisition (DIA) has been emerged as a powerful technology for untargeted metabolomics due to its capability to acquire all MS2 spectra and high quantitative accuracy. However, in DIA, the direct link between MS1 and MS2 ions in multiplexed MS2 spectra is missing, and there is no universal evaluation approach to decide whether MS1 or MS2 ion should be selected as the quantification ion for one metabolite. To address these current limitations in DIA data analysis, we developed a new strategy integrating DIA spectral library construction,quantitative ion selection with large-scale targeted re-extraction in DIA data itself. First, we proposed a new methodology that enabled us to construct a comprehensive spectral library directly from DIA data (referred as DIA-Lib, usually from pooled samples). The constructed DIA-Lib possessed the strengths including broad spectrum coverage, high quality and high reproducibility. Then, for every feature in DIA-Lib, the suitable quantitative ion was selected by Qscore method we proposed. We demonstrated the advantage of Qscore quantification method such as accuracy, linearity, and reproducibility. Furthermore, the high-coverage DIA-lib was used to re-extract ions in biological samples. Combining with the ion information selected by Qscore method above, large-scale metabolites extraction and accurate quantification in biological sample can be realized. Finally, the strategy was applied on colorectal cancer clinical sample set. Compared with conventional MS1 quantification method, our method performed better on separating cancer and adjacent tissues.

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*AWARD WINNERS

TECHNOLOGY

P-515 Rapid analytical platforms for biofluid profiling in discovery metabolomics

PRESENTING AUTHOR: ***Adam King, Waters corporation and Murdoch University, United Kingdom***

CO-AUTHORS: ***Ian D Wilson, Robert S Plumb, Paul D Rainville, Robert D Trengove***

Metabolic phenotyping has been widely employed in large epidemiological studies in the effort to discovery more about health and disease. UPLC and hybrid mass spectrometers have been essential tools in analyzing the matrices chosen for these studies. However, long acquisition times per samples (>15 mins) has meant large sets, typically employed in epidemiological and biobanking studies, can take days to analyze, ultimately putting strain on resources. Furthermore, this has led to sample acquisition being performed across multiple runs for the same data set, risking the development of batch effects when attempting to recombine the data. In order to address these issues, a suite of rapid profiling methods have been developed, reducing run time and solvent consumption by 75%. These methods were employed for the analysis of rodent urine and human plasma lipid extracts from breast cancer patients, using a Waters Acquity I-class UPLC system coupled to a Synapt G2-Si QTof mass spectrometer. Standard LC-MS methods for metabolite and lipid profiling using 2.1 mm i.d. columns were geometrically scaled, reducing the i.d., analysis times, injection volumes and mobile phase flow rate, while ultimately increasing the linear velocity. Each scaling demonstrated a preservation of the retention mechanism with relative retention times of probe compounds being maintained. Incorporating the ion mobility schema improved resolution of co-eluting ions, ultimately improving spectral clarity and with the generation of collisional cross section values, increased confidence in identification through database searches.

P-516 Sensomics: understanding flavor properties in food

PRESENTING AUTHOR: ***Brenda Ammerlaan, DSM Food Specialties, Netherlands***

CO-AUTHORS: ***Raymond Raymaker, Leon Coulier, John Gauvin, Peter Lankhorst, Adriana Carvalho de Souza, Cock Tas, Marjon Kok, Margriet Hendriks, Marieke Nijmeijer, Denise Jacobs, Maurien Olsthoorn***

DSM Food Specialties develops and sells process flavors. Understanding flavor at molecular level and the impact on sensory flavor perception, enables steering of the desired product properties during processing. This poster presentation outlines the workflow we have developed to identify mutual relations between metabolites and sensory attributes. Thirty different commercially available DSM process flavors were characterized both in sensory as well as chemically. For sensory evaluation, QDA (Quantitative Descriptive Analyse was done with a trained Savoury panel (n=12) on 46 attributes in odour, flavor and mouthfeel. To cover the broadest possible range of chemical compound classes, an untargeted sensomics approach was developed that combines four different complementary analytical methods to analyze the volatile and non-volatile metabolites that are possibly related to the flavor of our process flavors. The non-volatiles were analyzed by NMR and a GC-FID/MS method in combination with oximation and silylation. All volatiles were analyzed by an SPME-GC-FID/MS method. LC-MS was done for general profiling of peptides up to about 15 amino acids. Sample preparation and method development was done for all four analytical methods. Using these methods, all process flavors were characterized successfully. Multivariate data-analysis was applied to identify possible correlations between the chemical analytical data and sensory data. Visualization tools are optimized and together with DSM colleagues in Savory Ingredients, more detailed data mining is being done to be able to translate findings into answers to business questions. A holistic metabolic profiling was applied to develop models to describe flavor in our process flavors.

P-517 Rapid Evaporative Ionization Mass Spectrometry (REIMS) offers direct from sample mapping of faecal metabolites without sample preparation

PRESENTING AUTHOR: ***Petra Paizs, Imperial College London, United Kingdom***

CO-AUTHORS: ***Alvaro Perdones-Montero, James Kinross, Simon Cameron, Zoltan Takats***

Faecal metabolomics can provide a unique metabolic signature of intestinal function in a non-invasive manner and is crucial for the identification of diagnostic and therapeutic biomarkers in gut-related diseases. However, this relies on homogenous distribution of biomarkers within samples. Mass spectrometric imaging (MSI) is a key analytical tool for biomarker discovery, though most studies have concentrated on tissue analysis rather than faeces. Here, we present a high-throughput analysis pipeline using REIMS imaging, which allows for near-real time analysis and mapping of metabolites in whole fresh human faecal samples. Our strategy allows in-depth analysis of the diversity and complexity of the faecal metabolome by visualizing the relative abundance and spatial distribution of metabolites. This pipeline has been optimized and validated as a high-throughput tool for direct-from-sample analysis with minimal sample preparation: Whole faecal samples (<1 hour of bowel evacuation) were segmented into cross-sectional plates (5mm) and analyzed at 1 mm resolution in negative and positive ionization modes. Pre-processing of data and statistical analysis in R Studio (V1.0.44) allowed for targeted or untargeted analysis. Faeces from control patients with no known gastrointestinal disease were investigated and a detailed analysis of key features was performed. In the fatty acid region, we observed the most abundant peaks at m/z 255.25, 281.25, 279.25, 283.25, and 311.15 to be distributed heterogeneously. We are now looking at performing observational studies in colorectal cancer patients to investigate the spatial distribution of potential biomarkers.The mapping of metabolites through REIMS imaging demonstrates the first MSI technique for faecal sample analysis.

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*AWARD WINNERS

TECHNOLOGY

P-518 Impact of instrument, detection method, and statistical methods in mouse plasma metabolite profiling

PRESENTING AUTHOR: ***Lukas Kucera, Czech Centre for Phenogenomics, Institute of Molecular Genetics of the ASCR, v.v.i., Prague, Czec, Czech Republic***

CO-AUTHORS: ***Ashkan Zareie, Karel Chalupsky, Krystof Klima, Vendula Novosadova, Matthias Witt, Radislav Sedlacek***

Changes in the metabolic profile of plasma reflect the metabolic state of an organism and may reveal disease biomarkers or depict the phenotype of an animal model. Fast and reliable methods for high throughput are needed for non-targeted screening of samples. The aim of this study was the comparison of plasma metabolite measurements among a group of wild-type and Klk8 deficient mice across four methods; C18 HPLC column attached to an LC-MS instrument (6550 iFunnel Q-TOF, Agilent), MRMS instrument (7T, scimaX, Bruker) coupled with an ESI source (FIA - direct infusion) or MALDI, and MALDI-TOF-MS instrument (rapifleX, Bruker). Ions in positive polarity mode were acquired. Results were analyzed by Metaboanalyst statistical toolkit (Metaboanalyst 4.0, McGill Univeristy) and linear mixed-effects models (lme4 package, R language). Number of peaks identified by each instrument, detection method and number of significant peaks were scored by different statistical models. In positive polarity the MRMS instrument detected the highest number of peaks (6682 by FIA and 5246 by MALDI). For FIA method, Metaboanalyst toolkit found 366 peaks in positive mode, whereas mixed-effects model analyses reported 677 peaks. In conclusion, flow injection analysis (FIA) on the MRMS instrument followed by mixed-effect model analysis may aptly serve the purpose of fast metabolite profiling of plasma.

P-519 rMSIcleanup: an open-source computational tool for matrix-related peak annotation in MALDI-MSI

PRESENTING AUTHOR: ***Gerard Baquer Gómez, Universitat Rovira i Virgili, Spain***

CO-AUTHORS: ***Gerard Baquer, Pere Ràfols, Maria Garcia-Altares, Maria Vinaixa, Xavier Correig***

Laser Desorption/Ionization Mass Spectrometry Imaging (LDI-MSI) is a label-free technology that provides spatially resolved molecular information from tissue sections. LDI-MSI has been broadly adopted in proteomics and peptidomics. Its use in metabolomics has received much attention as a novel tool to understand mechanisms underlying complex diseases such as cancer or diabetes. However, conventional organic matrices used in MALDI-MSI cause spectral interferences in the low m/z range which seriously hamper metabolomics data processing. As an alternative, several matrix-free techniques such as sputtered metal nanolayer deposition have been used to significantly reduce such interferences. With the aim of expanding LDI-MSI usage to metabolomics applications we are developing rMSIcleanup, an open-source R package to annotate exogenous signals related to the ionization source (organic matrix or metal layer) based on its chemical composition. The development version of rMSIcleanup is available at https://github.com/gbaquer/rMSIcleanup. As the first validation, rMSIcleanup was challenged using images acquired with a sputtered silver nanolayer. Silver clusters previously reported in literature were consistently identified and chemically unfeasible cluster patterns showed lower similarity scores. A second validation step is in progress aiming to replicate the results using MALDI-MSI images. This includes a dataset containing images acquired using the most common MALDI matrices and both TOF and FTICR spectrometers. In conclusion, we have developed an open-source software tool that confidently annotates silver-related peaks in Ag-LDI-MS which anticipates its applicability to MALDI-MSI. Once demonstrated its performance in MALDI-MSI, rMSIcleanup will pave the path to the use of common organic matrices for metabolomics studies.

P-520 Discriminant analysis and feature selection in hyperspectral imaging using CORRS-CV

PRESENTING AUTHOR: ***Guillermo Quintas, Leitat Technological Center, Spain***

CO-AUTHORS: ***Juan Daniel Sanjuan, David Pérez-Guaita, Julia Kuligowski***

Biomarker identification through hyperspectral imaging is gaining popularity in the clinical field. Hyperspectral imaging generates large, complex datasets that require high-throughput data processing and data mining for biological analysis. The application of multivariate discriminant analysis using bilinear models such as PLS-DA to hyperspectral images requires to unfold the spatial directions in a two-way matrix, resulting in a loss of spatial information and structure. During model development, internal validation methods such as random k-fold cross-validation (CV) are widely used. However, a selection of the k subsets completely at random results in a loss of spatial structure and pixel-neighbourhood information. Highly correlated, spatially proximate pixels are distributed simultaneously into train and validation sets, leading to overly optimistic performance estimates. The use of biological replicates to define k-fold CV splits provides the most accurate estimation of the generalization performance, but it is inefficient when the number of replicates is scarce. Using simulated data sets as well as real IR and MS imaging data, we show the applicability of COnstrained Repeated Random Subsampling–Cross Validation (CORRS-CV). Results show that CORRS-CV avoids overly optimistic effects due to spectral oversampling in IR imaging. In situations where holding images back for testing is a waste of valuable information, CORRS-CV reduces the overly optimistic bias due to the use of test pixels close to the train set. Besides, the combined use of CORRS-CV and rank products increases the robustness of the selection of candidate biomarkers.

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TECHNOLOGY

P-521 Multi-metabolomics strategy sheds light on monoterpene indole alkaloids in Catharanthus roseus

PRESENTING AUTHOR: ***Ryo Nakabayashi, RIKEN CSRS, Japan***

CO-AUTHORS: ***Ryo Nakabayashi, Tetsuya Mori, Kei Hashimoto, Kiminori Toyooka, Yutaka Yamada, Hiroshi Tsugawa, Kazuki Saito***

Monoterpene indole alkaloids (MIAs) are one of important pharmaceutical resources in medicinal plants. For instance, 13.5% of Apocynaceae plants that produce MIAs were used for medicinal purposes in the world. Exploring known and unknown MIAs in natural plant resources is worthwhile in metabolomics. Here, we established an approach for efficiently finding MIAs in Catharanthus roseus, which is a MIA-producing plant (Apocynaceae). Metabolome data were acquired in an untargeted way using liquid chromatography-tandem mass spectrometry (LC-MS/MS) in 15N (nitrogen)-labeled and non-labeled Catharanthus. Principal component analysis using the 15N- and non-labeled MS data in the flowers, leaves, petioles, stems, and roots paired a set of non-labeled monoisotopic ion and 15N-labeled counterpart using retention time. Mass shift between the ions showed the number of N atom in the monoisotopic ions. In MS/MS analysis, the mass shift of the product ion m/z 144.08 that is derived from indolic skeleton was evaluated. Finally, the elemental composition of 45 MIAs was identified in the plant. Similarity analysis using MS/MS spectra showed the commonality or specificity in the spectra. Detail MS/MS analysis identified a vintage MIA that has been never found in Catharanthus as well as known MIAs such as ajmalicine, catharanthine, perivine, and yohimbine. Combined analysis of microscopy and imaging mass spectrometry characterized the localization of the newly identified MIA, suggesting a function in a certain tissue. The metabolome data will be uploaded to the PlaSMA database (http://plasma.riken.jp/) at an appropriate time.

P-522 Discovery of new cholesteryl esters using a mathematical model-assisted UHPLC-MS/MS method

PRESENTING AUTHOR: ***Jin-Lan Zhang, Institute of Materia Medica, CAMS & PUMC, China***

Cholesteryl esters (CEs) are composed of the 3-hydroxyl group of cholesterol and a fatty acyl chain through an ester bond. Abnormal CE levels are often related to various diseases, particularly hyperlipidemia and atherosclerosis. A mathematical model-assisted ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS) method has been developed to profile CEs in plasma, different density lipoprotein samples from humans, rats, and golden hamsters. 81 CE candidates were detected in the above samples, of which 24 CEs were reported in the Human Metabolome Database and 57 CEs were newly identified based on a created database of theoretically possible CEs. Three mathematical models based on the characteristic chromatographic retention behavior related to structural features were established and validated using commercial and synthetic CE standards. The mathematical model-assisted UHPLC-MS/MS strategy was proposed to provide a global profile and identification of CEs. With the efficient strategy, 74 CEs in plasma of golden hamster were identified and then quantified in normal and hyperlipidemic golden hamsters by dynamic multiple reaction monitoring (dMRM). 21 CEs among 35 shared potential biomarkers were newly found for hyperlipidemia. Our study will contribute to the in-depth study of CE functions and the discovery of biomarkers for diseases.

P-524 Using Knime for the analysis of LC-MS/MS metabolic datasets of amitriptyline and verapamil

PRESENTING AUTHOR: ***Nouf Alourfi, School of Chemistry, University of Bristol, United Kingdom***

A method for studying metabolic datasets has been developed by using Knime (Konstanz Information Miner). Knime is free and open-source data analytics, reporting and visualization platform. Our aim is to address the growing variety and complexity of data in this field and thus contribute to improving the analysis process in general. We demonstrate our approach using datasets derived from an ESI-LC-MS/MS analysis of in vitro metabolites of amitriptyline and verapamil using liver microsomes. Knime platform has been used to create, treat, share and store the raw ESI-LC-MS/MS data in a transparent and straightforward way. By using our approach, it was possible to automatically generate standard MS data visualisations. Moreover, we extend Knime by generating putative metabolites and MSMS spectra of our drugs using computational applications such as SyGMa and CFM-ID. SyGMa consists of a set of reaction rules covering a broad range of phase1 and 2 metabolism that has been derived from metabolic reactions demonstrated in the metabolite database which take a place in humans. Next, we used a Competitive Fragmentation Modeling which is used by CFM-ID to produces a probabilistic generative model for the MS/MS fragmentation process and machine learning techniques to adapt the model parameters from data. This generated model can be used to predict the spectra for chemical structure of a given drugs and then computes the predicted spectrum for each candidate and compares it to the input spectrum to rank the candidates metabolites according to how closely they match. Using this workflow, we are able to assign structures to candidate metabolites within our samples.

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TECHNOLOGY

P-525 Supporting reproducibility in metabolomics via a data analysis reporting template and the mzTab-M data standard

PRESENTING AUTHOR: *Reza Salek, IARC, France*

CO-AUTHORS: *1 Considine EC, Salek R., 2 N Hoffmann, J Rein, T Sachsenberg, J Hartler, K Haug, G Mayer, O Alka, S Dayalan, JTM Pearce, P Rocca-Serra, D Qi, M Eisenacher, Y Perez-Riverol, J Antonio Vizcaíno, RM. Salek, S Neumann, AR. Jones*

A significant challenge in metabolomics is result reproducibility and confidence in metabolite identification, which are directly related to the choice of analytical instruments, available evidence extracted through data analysis, and quality of the reporting. Here two approaches are presented to improve reproducibility and reporting in metabolite identification. To enable comprehensive data analysis reporting, we present an R markdown reporting template [1] that guides the production of text and generates workflow diagrams based on user input. This R Markdown template presents a set of minimum information requirements specifically for data pre-treatment and data analysis in biomarker discovery (https://github.com/MSI-Metabolomics-Standards-Initiative/MIDAS). We will also present mzTab-M, a Data Standard for Sharing Quantitative Results in Mass Spectrometry Metabolomics [2]. This standard was developed as a joint effort between the Metabolomics Standards Initiative and Proteomics Standards Initiative organisations over several years. mzTab-M is a simple tab-separated text-format, with highly standardized structures and a detailed specification document, tightly coupled to a validation software API, and mandatory controlled vocabulary terms to populate it. The format represents final quantification values from analyses, as well as the evidence trail in terms of features measured directly from MS (e.g., LC/GC-MS, DIMS, etc.) and the approaches used to identify molecules. It allows clear communication of identification ambiguity to readers and has widespread adoption potential, for details and implementations see https://github.com/HUPO-PSI/mzTab. Both approaches presented will facilitate more transparent and reproducible metabolomics. Citation and details: [1]Metabolites2019.doi:10.3390/metabo9030043. [2]Anal Chem.2019.doi:10.1021/acs.analchem.8b04310.

P-526 L-homocysteine Sulfinic Acid and Cysteic Acid as Novel Biomarkers of Acute Myocardial Infarction

PRESENTING AUTHOR: *Youngja Park, Korea University, South Korea*

CO-AUTHORS: *Adnan Khan*

Identifying changes in serum metabolites before occurrence of acute myocardial infarction (AMI) is an important approach for finding a novel biomarker of AMI. In this retrospective cohort study, serum samples obtained from patients at risk of AMI risk (n = 112) and non-risk control (n = 89) were tested using high resolution metabolomics (HRM), coupled with LC-MS/MS. Partial least-squares discriminant analysis (PLS-DA), along with univariate analysis using false discovery rate (FDR) at q = 0.05, were performed to discriminate metabolic profiles and to determine significantly different metabolites between healthy control and AMI risk groups. PLS-DA significantly separated the AMI risk sera from healthy. Altered metabolic pathways analysis in KEGG online database showed that biosynthesis of amino acid, 2-ococarboxylic acid metabolism, tryptophan metabolism, and amino sugar and nucleotide sugar metabolism were mainly altered in AMI risk sera. The metabolites associated with these pathways were mainly elevated among AMI patients. Further validation and quantifications by MS/MS showed that carnitine, L-homocysteine sulfinic acid (L-HCSA), and cysteic acid (CA) were upregulated, while L-cysteine and L-cysteine sulfinic acid (L-CSA) were downregulated, specifically among AMI risk sera. Additionally these discriminant metabolic profiles and phenotypes among AMI were not related to other factors such as hypertension, smoking or alcoholism. In conclusion our study suggests that detection of upregulated L-HCSA and CA along with carnitine among AMI risk patient could serve as promising non-invasive biomarkers for early detection of AMI.

P-527 Establishing a Spectral Library and Accurate Mass Retention Time (AMRT) Database for Neonatal Metabolomics Analysis

PRESENTING AUTHOR: *Anas Kamleh, Thermo Fisher Scientific, Sweden*

CO-AUTHORS: *Chiara Lavarello, Sebastiano Barco, Igor Fochi, Martina Batolucci, Gino Tripodi, Giuliana Cangemi, Andrea Petretto*

Metabolomics is an established discovery tool for biomarker discovery, disease diagnosis and novel mechanistic insights of pathophysiological processes. Liquid-chromatography mass spectrometry (LCMS) is currently the method of choice for metabolome analysis, however, the wide variety of chromatographic columns and conditions is presenting a challenge in sharing and interpretation of metabolite identities. The metabolic standard initiative (MSI) guidelines require identification of metabolites to be based on matching accurate mass, retention time and fragmentation spectra to those from authentic standards. In order to facilitate sharing of information between groups involved in neonatal metabolomics analysis, we have established a spectral library of compounds in the commercial product (IROA) metabolite identification. Data in the spectral library was acquired using flow injection analysis (FIA) which allowed the fast acquisition of spectra at a wide range of collision energies (0-120, normalized collision energy NCE). Additionally, a separate library, with optimized collision energy, was constructed by applying rules of maximal selectivity and structural information. The latter library is the basis of further confirmation of the identity of compounds in future studies. Furthermore, we provide a scheme for obtaining the minimum number of metabolite mixtures that provide full coverage of the IROA kit. These mixtures were used to establish an accurate mass/retention time (AMRT) databases using six different chromatographic columns (three reversed-phase and three HILIC) and chromatographic suitability criteria were applied to select the most appropriate methods. Metabolite identification in matrices (plasma, urine and sweat) were further validated using the established AMRT databases and libraries.

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TECHNOLOGY

P-528 Metabolite identification from LC-MS/MS spectra using deep learning

PRESENTING AUTHOR: ***Svetlana Kutuzova, Technical University of Denmark, Denmark***

CO-AUTHORS: ***Douglas McCloskey, Christian Igel***

Mass spectrometry is a powerful high-throughput technology for chemical composition assessment. However the data processing of the resulting spectra is a major bottleneck for large studies, and in particular the metabolite identification from the mass spectra. The joint community effort of collecting and maintaining metabolomics spectral databases provides the opportunity to approach the metabolite identification problem with powerful but data-hungry algorithms including deep learning. We present a novel deep learning based algorithm for compound identification that makes a prediction of a structural chemical fingerprint based on a LC-MS/MS spectrum of a compound. Both raw spectra and fragmentation tree predicted by SIRIUS software are used as an input. A Tree-LSTM network is used to process the fragmentation trees alongside with a feed forward neural network that captures patterns in the spectral data. Our method is validated on the CASMI 2017 challenge dataset. While the method does not yet outperform the state-of-the-art approach it is shown to be a proof of concept and a solid base for future developments. The future work would include learning fragmentation rules from the spectrum itself enabling a complete end-to-end spectrum analysis.

P-529 Prediction Models of Retention Indices: Application to Gas Chromatography Coupled with High Resolution Mass Spectrometry for two Column Types: DB-624 and HP-5ms

PRESENTING AUTHOR: ***Adrian Haiduc, PMI, Switzerland***

CO-AUTHORS: ***E. Dossin, P. Diana, P.A. Guy, N.V. Ivanov, M. Peitsch***

Monitoring of volatile and semi-volatile compounds was performed using gas chromatography (GC) coupled to high resolution electron ionization mass spectrometry, using both headspace and liquid injection modes on DB-624 and HP-5ms columns. A total of 1'300 reference compounds (n=400 analyzed on HP-5ms and n=900 on DB-624 columns), including n-alkanes (covering C5 to C30) as reference index markers, were analyzed and experimental linear retention indices (LRI) were determined. These reference compounds were randomly split into training and validation sets. LRI for all 1'300 reference compounds were predicted based upon computational Quantitative Structure-Property Relationship (QSPR) models using calculated 2D descriptors, and based on multiple approaches: PLS, Lasso regression, stepwise MLR, Genetic Evolution Algorithm predictor selection and Neural Networks, with PLS and Lasso providing the fastest calculation and most accurate prediction level. Correlation coefficients for experimental versus predicted LRI values were calculated at 0.96 for DB-624 and 0.98 for HP-5ms for the training sets and at 0.94 and 0.95 for the validation sets, respectively. These models were then used to predict LRI values for several thousand reported metabolite compounds. The predicted LRI values can be used for column type selection as well as increased confidence level in unknown identification by means of the Mahalanobis distance.

P-530 Synthesis of IROA fragmentation scans from recursive DDA fragmentation data

PRESENTING AUTHOR: ***Chris Beecher, IROA Technologies, United States***

CO-AUTHORS: ***Felice de Jong, Alexander Raskind, Philip L. Lorenzi, Lucas J. Veillon***

IROA fragmentation patterns are unique in two significant ways: 1) the ability to discriminate between fragment and artifact peaks in the MS-MS spectra, and 2) the formula for every fragment is known. However, the acquisition of IROA fragment patterns has always required the use of fragmentation windows wide enough to include the entire IROA peak cluster, but ideally no wider. Since the width of each IROA peak cluster is determined by the number of carbons in the molecule, no single window width setting can be used. Using recursive DDA systems such as Thermo's AquireX, or Agilent's iDDA, have made it possible to acquire fragmentation scans for a majority of the individual peaks of an IROA cluster and then sum these fragmentation scans to synthesize what the wide window MS-MS scan would have shown. In this poster we demonstrate the implementation of such a strategy. The synthesized scans may be processed to exclude artifact peaks and determine formulae for all fragments. These two factors provide for better compound identification, and structure elucidation if the identity of a peak is unknown. While dot product calculations can still be performed on the synthetic scans, the availability and use of fragment formulae is cleaner and more accurately verifies compound identity. We believe that this is the first use of the systematic synthesis of complex MS-MS scans from individual MS-MS scans. We expect that many related, derivative applications will soon appear.

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TECHNOLOGY

P-531 Extractive Ratio Analysis NMR Spectroscopy for Improved Metabolite Identification in Complex Biological Mixtures

PRESENTING AUTHOR: ***Dan Raftery, University of Washington, United States***

CO-AUTHORS: ***G.A. Nagana Gowda, Liladhar Paudel***

The complexity of biological mixtures continues to challenge efforts aimed at unknown metabolite identification in the metabolomics field. To address this challenge, we provide a new method to identify related peaks from individual metabolites in complex NMR spectra. Extractive ratio analysis NMR spectroscopy (E-RANSY) builds on our previously described ratio analysis method which calculates a peak ratio divided by its standard deviation [Anal. Chem. 2011, 83, 7616-7623], such that peaks from the same molecule are enhanced and peaks from other metabolites are diminished. Here, E-RANSY first creates simplified NMR spectra that result from liquid-liquid extraction of metabolites under varied pH conditions. Under such conditions, metabolites from the same biological specimen are extracted differentially such that their signals vary dramatically across the sample set; the resulting NMR spectra exhibit characteristics favorable for unraveling unknown metabolite peaks using ratio analysis. We demonstrate the utility of the E-RANSY method for the analysis of carboxylic acid containing metabolites in human urine. E-RANSY performs better than correlation methods such as STOCSY as well as the original RANSY method, and offers new avenues to identify unknown metabolites in complex biological mixtures. We will also discuss the development and optimization of the algorithm for RANSY and ways to expand its utility, such as using it to deconvolute MS data and for bridging the gap between NMR and MS spectra of the same sample for improved unknown detection.

P-532 Advantages and limitations of Orbitrap GC-MS in metabolomics research

PRESENTING AUTHOR: ***Daniel Stettin, Friedrich-Schiller-University Jena, Germany***

CO-AUTHORS: ***Georg Pohnert***

The annotation of unknown compounds represents the current bottleneck in MS-based metabolomics research (Viant et al., 2017). LC-MS based metabolomics has received a boost in that regard with the introduction of the Q-Exactive Orbitrap MS (Alvarez-Rivera et al., 2019). The later introduced Q-Exactive Orbitrap GC-MS has remained fairly unexplored in its potential for metabolomics research. In this work, we set out to assess what level of insight into the metabolome of a non-model organism the GC-Orbitrap can provide. We devised a simple comparative metabolomics experiment involving an osmotic stress treatment on a unicellular algae and ran the samples both on a nominal mass GC-MS as well as the GC-Orbitrap. Resulting data was compared in regards to data quality, degree of putative annotation and the potential to identify unknowns. Herein we show that the accurate mass provided by the GC-Orbitrap does not improve compound annotation when combined with traditionally used EI-database matching but does enhance current annotation techniques (Lai et al., 2017). We successfully identify more than 50% of the dysregulated compounds found on both platforms. Still, the majority of unknowns remain unannotated despite the plethora of structural information provided by the GC-Orbitrap. These results show the potential of the Orbitrap mass analyzer for GC-MS based metabolomics but also reveal a lack of computational support currently hampering the rapid annotation of unknowns.

P-534 Differentiation of positional isomers of drug metabolites using infrared ion spectroscopy

PRESENTING AUTHOR: ***Rianne van Outersterp, FELIX Laboratory, Netherlands***

CO-AUTHORS: ***Giel Berden, Valerie Koppen, Filip Cuyckens, Jos Oomens, Jonathan Martens***

An understanding of the metabolism of drug candidates, including the identification of downstream drug metabolites, is a crucial step in drug development. Due to its high sensitivity and selectivity, mass spectrometry (MS) is often the analytical method of choice. However, closely related compounds may be hard to distinguish using (tandem) MS alone, as these are often isobaric and give identical fragmentation mass spectra. Alternative methods involving expensive and time-consuming purification steps are usually needed to confidently resolve full molecular structures. Infrared ion spectroscopy (IRIS) records an IR spectrum of a mass-selected gas-phase ion directly inside a mass spectrometer. The IR fingerprint is highly sensitive to molecular structure, and can be recorded for each m/z-feature detected in a standard MS experiment. We exploit this technique, which has full MS sensitivity and compatibility, to identify small molecules resulting from untargeted MS-based experiments, including drug metabolites. Here, we explore the use of IRIS for the differentiation of positional isomers resulting from the phase I metabolism reactions (usually oxidation, reduction or hydrolysis) that introduce a reactive or polar group on a phenyl ring in a drug molecule. The chemical modification that occurs can usually be determined from the mass spectrum, but the exact site of biotransformation often remains unknown. We demonstrate how metabolites bearing a ortho-, meta- or para-hydroxylation can be distinguished based on their IR spectra. Also, we show that identification is possible on the basis of spectra predicted by quantum-chemical calculations, opening opportunities for reference-standard free identification.

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TECHNOLOGY

P-535* DynaStl: a dynamic database for LC-MS annotation in metabolomics

PRESENTING AUTHOR: ***Santiago Codesido, Université de Genève, Switzerland***

CO-AUTHORS: ***Giuseppe Marco Randazzo, Fabio Lehmann, Víctor González-Ruiz, Arnaud García, Ioannis Xenarios, Robin Liechti, Alan Bridge, Julien Boccard, Serge Rudaz***

One of the key problems in metabolomics is identification from few analytical parameters, within the wide range of compounds that comprises the metabolome. Steroidomics is a salient example of this problematic, facing the additional difficulty of dealing with a set of molecules with very similar structures, and so they are the perfect testing ground for new approaches. Liquid chromatography (LC) is often the method of choice for metabolite separation, but such structural similarity can be compromising for this separation due to the consequently similar physico-chemical properties. The problem is usually overcome by careful tuning of the mobile phase gradient, to focus the separation on compounds of interest. However, in a standard workflow this is highly problematic for annotation. It requires characterizing a library of known compounds for every fine-tuned configuration. We present a software solution, DynaStl, capable of annotating LC-MS (liquid chromatography-mass spectrometry) features by dynamically generating the retention times from a database containing intrinsic properties for a library of metabolites. In this way, the chemical characterization of the library only needs to be performed once, and the generated retention times are adapted to the parameters of each gradient on the fly. We study the influence of experimental vs. in-silico compound properties on the quality of the prediction and the annotation, and we introduce a calibration mechanism to increase accuracy and compensate for deviations in the input parameters. We run tests on both standards and real samples, and observe that the algorithm produces reliable predictions, suitable for metabolomics compound annotation.

P-536 Comparison of the metabolite profiles of human and canine saliva

PRESENTING AUTHOR: ***Soile Rummukainen, School of Pharmacy, Faculty of Health Sciences, University of Eastern Finland, Finland***

CO-AUTHORS: ***Jenni Puurunen, Olli Kärkkäinen, Seppo Auriola, Arja M Kullaa, Hannes Lohi, Kati Hanhineva***

There is a growing interest towards using saliva as a non-invasive sample material for monitoring health and disease status to assist diagnosis or studying molecular mechanisms of disease pathologies. Same applies to domestic dogs (Canis lupus familiaris) which suffer from similar diseases like humans having e.g. diabetes, inflammatory bowel disease and cancer. Human saliva is known to be rich in small molecules, but there is no data available for dog saliva metabolome even though a dog is also a modern model for human diseases. The present study investigated metabolite composition of stimulated saliva samples collected from 13 privately-owned dogs and 14 human individuals. We applied non-targeted metabolomics method based on ultra-high performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry. Complementary methods, reverse phase and hydrophilic interaction chromatography were used with both, positive and negative ionization, to cover a broad repertory of compounds. Metabolite identification was accomplished using MS-DIAL software and both in-house and open-source libraries. With this approach, > 200 metabolites were identified revealing distinct metabolic profile in dog and human saliva. The biggest differences between species were found among lipids and small peptides. Other identified metabolites were amino acids, amino acid derivatives, biogenic amines, nucleic acid subunits, organic acids and other metabolites, such as general waste products. In addition, some exogenous compounds were identified. Further studies are needed to evaluate the utility of saliva as a diagnostic material for canine health monitoring with possibilities to benefit dog as well as human health in the future.

P-537 Quantitative Analysis of Over 600 Metabolites in NIST SRM 1950 Using Multiple Analytical Platforms

PRESENTING AUTHOR: ***Rupasri Mandal, University of Alberta, Canada***

CO-AUTHORS: ***Jun Han, Paulina de la Mata, Xian Luo, Meera Shanmuganathan, Pascal Mercier, Rene Zahedi, Michael Overduin, James Harynuk, Philip Britz-McKibbon, Liang Li, Christoph H. Borchers, and David S. Wishart***

A wide variety of analytical methods have been developed for targeted metabolomics. By combining multiple techniques, it is now possible to achieve much more comprehensive coverage of a sample's metabolome. However, the precision, accuracy, and level of metabolome coverage of different platforms is often not well known. To answer these questions we chose to comprehensively characterize a widely-studied biofluid sample (human pooled plasma, NIST Standard Reference Material, SRM 1950) using 12 different targeted assays conducted on 8 different analytical platforms. The platforms included NMR (700 and 800 MHz), direct injection/liquid chromatography tandem mass (QTrap) spectrometry (DI/LC-MS/MS), LC-MS with isotope-labeled internal standards (LC-MRM-MS), LC–coupled with high-resolution mass (Orbitrap) spectrometry (LC-HRMS), inductively coupled plasma mass spectrometry (ICP-MS), two-dimensional gas chromatography mass (QTOF) spectrometry (GCxGC-TOF MS) and capillary electrophoresis with ultraviolet (CE-UV) and mass spectrometry (CE-MS). A total of 860 quantitative measurements for 628 metabolites were obtained from the 12 different analytical assays. Another 81 metabolites are identified (but not quantified). For metabolites quantified by more than one method, the measured concentrations were compared between methods and against either NIST reference data or known reference ranges from the literature. Most assayed metabolites showed excellent cross-platform agreement (10-15%). Clear differences in platform coverage and sensitivity are evident. This represents the most complete quantitative characterization of SRM 1950. It also provides high-confidence reference values for SRM 1950 that should allow other research labs to calibrate their assays. Furthermore, this work gives important insights into the strengths and weaknesses of different metabolomic platforms/assays for plasma/serum analysis.

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*AWARD WINNERS

TECHNOLOGY

P-538 Machine-learning based spectral similarity measures to better identify different yet related compounds from large metabolomic datasets.

PRESENTING AUTHOR: *Florian Huber, Netherlands eScience Center, Netherlands*

CO-AUTHORS: *Justin J.J. van der Hooft, Simon Rogers, Lars Ridder*

Extensive high-throughput mass spectrometry has become an important tool in many areas of life sciences and medicine. Analyzing and interpreting the resulting complex mass spectral data remains a challenging task, in particular for mixtures containing large numbers of unidentified compounds. One key challenge in extracting useful information from such data is to determine if spectra belong to identical or similar molecules. This is typically done by deriving spectral similarity scores, currently often based on comparing (intensities for matching) peak positions, for instance by calculating a modified cosine score (as used in GNPS molecular networking). Those measures work well for spectra obtained for very similar compounds, but often perform poorly when used to find similarities between spectra of notably different yet related compounds. This is assessed by using Tanimoto coefficients between molecular fingerprints from a large set (>10,000) of MS/MS reference spectra as benchmark. We here propose a number of alternative approaches for measuring spectral similarity which are based on established machine-learning algorithms including techniques adapted from natural language processing, but also PCA and deep autoencoders. We will present several measures that outperform the modified cosine score in selecting spectra from structurally closely related molecules in datasets containing potentially unknown compounds. We further find that some of the presented measures show complementary characteristics which can either be combined or be used to address different types of similarity. Taken together, we conclude that these novel spectral similarity measures are a promising alternative for established measures.

P-539 SIRIUS 4 - Turning tandem mass spectra into metabolite structure information

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CO-AUTHORS: *Kai Dührkop, Markus Fleischauer, Martin A. Hoffmann, Juho Rousu, Sebastian Böcker*

Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) is one of the predominant experimental platforms for untargeted metabolomics, but searching acquired tandem spectra in spectral libraries will only identify a small portion of the measured metabolites. Here, we present the new release of the SIRIUS software. SIRIUS 4 is the best-in-class software method for de novo molecular formula annotation and structure elucidation. SIRIUS 4 integrates high-resolution isotope pattern analysis and fragmentation trees for molecular formula identification. CSI:FingerID is seamlessly integrated via a RESTful webservice to search MS/MS spectra in a molecular structure database. SIRIUS 4 has a novel isotope pattern scoring. Using a deep neural network, SIRIUS detects rare elements from the isotope pattern. The running times of SIRIUS were reduced by more than two orders of magnitude. CSI:FingerID is now supporting negative ion mode spectra and integrates new kernels and fingerprints for a better identification performance. Users can search in custom suspect databases or view the substructure recommendations for manual structure elucidation. In evaluation on the Agilent MassHunter database the number of correct molecular formula annotations solely based on the isotope pattern increased by 74.3% compared to predecessor SIRIUS 3. On 208 compounds from the CASMI 2016 challenge, SIRIUS 4 correctly identified the molecular formula in 93.75% of the cases. Out of the 127 compounds in positive ion mode CSI:FingerID correctly identified 57.5% of the structures when searching in PubChem and 74% when searching in a smaller database of 0.5 million structures of biological interest. The best competitor correctly identified 36.22%.

P-540 Integrated NMR and LC-MS based metabolomics approach for biomarker identification for radiation exposure

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CO-AUTHORS: *Kiran Maan, and Poonam Rana*

In the event of an intentional or accidental release of ionizing radiation in a densely populated area, timely assessment and triage of the general population for radiation exposure is critical. Despite decades of research, counter measures still lack. In this study, we describe the potential of integrated NMR and LC-MS approaches in evaluating the radiation biomarkers. Untargeted profiling by means of broad-spectrum, highly sensitive, UPLC-ESI-QTOFMS provides a comprehensive list of metabolites at one go in a single biofluid. Present study aims to discover new, as well as validate the previously identified metabolic signatures for whole-body irradiation in mice. The study comprised 33 C57BL6 male (8-10 weeks) mice distributed as 5Gy, 7.5Gy and controls having 11 each and irradiated through60Co gamma source. Urine samples collected post24 hrswere run in both ESI positive and negative mode. All the data were normalized by sum and were than pareto-scaled followed by multivariate analysis including PCA and PLS-DA. Of the total 1514 (positive) and 1764 (negative) peaks univariate analysis(t-test, $p<0.05$significant) revealed a total of 658 significant (positive) molecules with creatinine ($p=9.8x10-5$) and L-carnitine ($p=3.6x10-8$)from 5Gy whereas betaine ($p=2.0x10-4$), 8-hydroxyquinone ($p=9.6x10-5$) and L-carnitine ($p=3.4x10-5$) from 7.5 Gy. Out of 537 significant (negative) molecules taurine (4.7x10-4) and Quinnolinic acid (6.3x10-4) were from 5Gy. Present study thus validates our previously (NMR) reported significant metabolites citric acid, hippuric acid and taurine. The results thus lay foundation for high-througput triaging by metabolomic biomarkers for effective medical management. Further pathway analysis also reveled results.

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TECHNOLOGY

P-541 ASICS: identification and quantification of metabolites in complex 1D 1H NMR spectra

PRESENTING AUTHOR: *Gaëlle Lefort, INRA, France*

CO-AUTHORS: *Laurence Liaubet, Hélène Quesnel, Cécile Canlet, Nathalie Vialaneix, Rémi Servien*

1H Nuclear Magnetic Resonance (NMR) is a high-throughput technology that allows to obtain metabolomic profile from easy-to-obtain fluids (such as blood) at moderate cost. It is thus a promising tool to detect practically usable biomarkers. However, its interpretation can be hard to make, because metabolites present from the 1H NMR spectrum of a complex mixture are often identified and quantified only from expert knowledge. To facilitate the use of such data, we developed a new R package, ASICS, that implements a method for the automatic identification and quantification of metabolites in 1H NMR spectra. The package combines all the steps of the analysis (management of a reference library with pure metabolite spectra, preprocessing, quantification, diagnosis tools to assess the quality of the quantification, post-quantification statistical analyses). The latest developments allow to improve alignment algorithm and quantification using information coming from all spectra of the same study. To assess the performance of ASICS, data from PORCINET (ANR-09-GENM-005) were used. Both the quantification and its impact on a post-quantification differential analysis were evaluated. Correlations between ASICS relative quantifications and biochemical dosages of three metabolites were computed and a similar analysis was performed with other quantification methods like Autofit or batman. These comparisons showed that ASICS allows for a faster and simpler direct biological interpretation than the classical bucket approach and obtains more precisely identified and quantified metabolites than other quantification methods. ASICS is released as an R/Bioconductor package.

P-542 High accuracy of retention time prediction for plant food bioactives using PredRet

PRESENTING AUTHOR: *Claudine Manach, INRA, France*

CO-AUTHORS: *Low D, Micheau P, Abranko L., Bronze M., Hanhineva K., Koistinen V., Stanstrup J, Manach C*

Plant food bioactives receive widespread interest for their protective health effects. However, due to their huge chemical diversity and the lack of chemical standards for many of their phase I, –II and microbial metabolites, their identification in untargeted metabolomics profiles of food or biofluids remains a challenging feat. Retention time (RT) is a valuable information for assisting the identification as it helps to narrow the number of hypotheses within an observed RT window to a manageable number of compounds to purchase or synthesize for confirmation. In the framework of the COST Action FA1403 POSITIVe (https://www6.inra.fr/cost-positive, we evaluated the usefulness of PredRet (http://predret.org), an open access RT database, to predict RT of plant food bioactive metabolites in a multi-laboratory test involving 18 laboratories and 24 reversed-phase Chromatographic Systems (CS=column + elution phases and gradient). Participants shared datasets of RT in their own CS for 29 to 104 compounds, covering a total of 471 chemicals, including highly polar to lipophilic aglycones, glycosides, conjugated and microbial metabolites of flavonoids, phenolic acids, alkaloids, and others. Depending on its comparability with other CSs, every platform obtained predicted RTs for 67 to 667 compounds that were not analysed in their conditions. The predictions were very accurate, with a median prediction error ranging from 0.03 to 0.76 min. Such level of prediction allowed distinguishing isomeric compounds. It also provided information in all CSs for rare standards. In conclusion, RT prediction with PredRet has proven very useful to facilitate annotation of plant food bioactives in metabolomics studies.

P-543 Metabolic effects of dietary glycerol supplementation in muscle and liver of European seabass and rainbow trout by 1H NMR metabolomics

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CO-AUTHORS: *Ludgero C Tavares, João Rito, Luís F Henriques, Paulo Rema, Leonardo J Magnoni, Ivan Viegas*

The sustainable development of aquaculture is still dependent in the substitution of fishmeal for alternative ingredients, especially for carnivorous fish, such as rainbow trout (Oncorhynchus mykiss) and European seabass (Dicentrarchus labrax). Glycerol has been already used as an alternative energy source in diets for farmed animals, sparing amino acids to other functions such as growth. The aim of the work was to evaluate the effects of dietary glycerol supplementation in rainbow trout and European seabass muscle and liver metabolome. Fish were fed diets with 0%, 2.5% and 5% glycerol, muscle and liver were collected, and tissue aqueous fraction was extracted. 1H-Presat and CPMG spectra were acquired for each muscle and liver sample, respectively, on a Varian VNMRS 600 MHz spectrometer. Both untargeted and targeted approached were followed applying Principal Component Analysis (PCA) and univariate statistical analysis, respectively. PCA plot scores showed scarce differences in muscle and liver metabolite composition in both species, regardless of the treatment. Regarding univariate analysis, European seabass had more variations in the muscle and liver metabolome than rainbow trout. European seabass presented changes generally related with protein biosynthesis pathways, while in rainbow trout variations were associated with choline-related metabolism. Albeit rainbow trout seems to be more suitable to be fed with these dietary glycerol percentages, the tested diets have the potential to be used in aquaculture production. NMR-Metabolomics approach proved to be adequate to be applied in these studies, providing a quick global overview of the results and also enabling the general tissue metabolite profiling.

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TECHNOLOGY

P-544 Accelerating Substructure Annotations in Complex Metabolite Mixtures

PRESENTING AUTHOR: ***Justin J.J. van der Hooft, Bioinformatics Group, Dept of Plant Sciences, Wageningen University, Netherlands***

CO-AUTHORS: ***Madeleine Ernst, Sam Stokman, Cher Wei Ong, Lars Ridder, Stefan Verhoeven, Ricardo da Silva, Mingxun Wang, Kyo Bin Kang, Joe Wandy, Pieter C. Dorrestein, Marnix H. Medema, Simon Rogers***

Deciphering complex metabolite mixtures remains a challenging task. Key reasons for this are the complexity and sheer number of information-rich mass fragmentation spectra hampering quick dereplication of known molecules and prioritization of novel chemistry. Here, however, we will take advantage of this information-richness by using computational approaches that discover spectral similarities and mass spectral patterns corresponding to the biochemical building blocks of molecules here termed substructures. We highlight recent advances that integrate existing metabolome mining tools with annotation tools such as Network Annotation Propagation as well as tools that specifically annotate possible peptidic spectra. This eases the interpretation of large mass spectral molecular networks. For example, we used the ClassyFire chemical classification ontology to annotate molecular families (MFs) observed in large MS/MS data collections. This could help to prioritize relevant MFs. In bacterial data, we could quickly assess how many peptidic MFs were present. New structural variants of the cyclic peptide Xenoamicin were found and Xenoamicin-related MFs were discovered by the substructures (spectral patterns) they shared. Additionally, an aminosugar substructure motif revealed many potentially novel natural products. Furthermore, integration of the discovery of mass spectral motifs by MS2LDA with automatic substructure annotation using MAGMa supports and accelerates substructure annotations. Finally, the introduction of MotifDB, a repository for annotated spectral patterns (Mass2Motifs) from MS2LDA, reinforces substructure discovery by capturing community knowledge that will be reused by seeding MS2LDA with structurally annotated Mass2Motifs. In conclusion, substructure annotation is a promising avenue to overcome challenges of metabolite annotation in complex metabolite mixtures.

P-545 Automated analysis of large-scale NMR data generates metabolomic signatures and links them to candidate metabolites

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CO-AUTHORS: ***Mattia Tomasoni, Mirjam Mattei, Roger Mallol Parera, Reyhan Sonmez, Daniel Krefl, Rico Rueedi, Sven Bergmann***

Identification of metabolites in large-scale 1H NMR data from human biofluids remains challenging due to the complexity of the spectra and their sensitivity to pH and ionic concentrations. In this work, we test the capacity of three analysis tools to extract metabolite signatures from 968 NMR profiles of human urine samples. Specifically, we studied sets of co-varying features derived from Principal Component Analysis (PCA), the Iterative Signature Algorithm (ISA) and Averaged Correlation Profiles (ACP), a new method we devised inspired by the Statistical total correlation spectroscopy (STOCSY) approach. We used our previously developed metabomatching (Rueedi et al. 2017) method to match the sets generated by these algorithms to NMR spectra of individual metabolites available in public databases. We concluded that both ISA and ACP can robustly identify about a dozen metabolites, half of which were shared, while PCA did not produce any signatures with robust matches.

P-546 Learning from Experience: Non Trivial Issues and Solutions in LC-MS- Based Metabolomics Analysis

PRESENTING AUTHOR: ***Ilana Rogachev, The Weizmann Institute of Science, Israel***

CO-AUTHORS: ***Maxim Itkin, Adam Jozwiak***

It is likely that most if not all metabolomics specialists employing LC-MS-based technology encounter samples displaying un-predictable behavior in the LC column or during MS ionization. While plenty of troubleshooting information is provided by instrument vendors they mostly concern with common LC-MS problems. Quite the reverse, solutions for uncommon problems originating from specific extracts or metabolites is barely accessible. In this presentation, I have composed a set of non-standard problems, engaged in our lab following more than 15 years of research in the HR LCMS-based metabolomics field. Examples of such challenges include retention time shifts of metabolites in the presence of highly abundant compounds, absence of molecular ion in the MS chromatogram, and more. Based on our past experience, I will provide several solutions and furthermore tips for predicting potential technical hitches prior to experimentation.

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TECHNOLOGY

P-547 Investigation of Microalgae Polar Lipidome by Optimized Chromatographic Separation, High-Resolution Mass Spectrometry, and Comprehensive Identification with Lipostar

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The polar lipidome of freshwater microalgae has been already investigated [1], mostly by liquid-chromatography (LC) coupled to high-resolution mass spectrometry (HRMS) [2]. However, studies focused on method development for the chromatographic separation of glycosylglycerolipids, i.e. sulfoquinovosyldiacyl- and sulfoquinovosyl-monoacyl-glycerols, (SQDG, SQMG), monogalactosyldiacyl- and monogalactosylmonoacyl-glycerols, (MGDG, MGMG), digalactosyldiacyl- and digalactosylmonoacyl- glycerols (DGDG, DGMG), are lacking. Due to glycosylglycerolipids high abundance in microalgae [1], in this study, a chromatographic optimization of both glycosylglycerolipids and phospholipids was carried out for the comprehensive characterization of spirulina microalgae lipidome. A Kinetex-EVO C18 column was used on a ultrahigh-performance LC system coupled by electrospray ionization source to a hybrid quadrupole orbitrap HRMS. Firstly, different chromatographic conditions were evaluated based on resolution, peak capacity, and peak shape, on a mixture of lipid standards. The optimized conditions were used for the analysis of spirulina microalgae. HRMS and MS/MS spectra were analyzed by Lipostar software [3]. Lipostar was implemented in order to both improve the identification of phospholipids and to allow the identification of glycosylglycerols, resulting in the identification of 205 lipids [4]. Finally, a targeted method was developed for the selective enrichment of SQDG and SQMG from spirulina microalgae, increasing the number of identified compounds. References: [1] E.Da Costa, J.Silva, S.H.Mendonça, M.H.Abreu and M.R.Domingues. Mar. Drugs 2016, 14(5), 101; [2] Y.H.Rustam, G.E.Reid. Anal. Chem. 2018, 90(1), 374−397; [3] L.Goracci, S.Tortorella, P.Tiberi, R.M.Pellegrino, A.Di Veroli, A.Valeri, G.Cruciani. Anal. Chem. 2017, 89(11), 6257−6264; [4] G.La Barbera, M.Antonelli, C.Cavaliere, G.Cruciani, L.Goracci, C.M.Montone, S.Piovesana, A.Laganà and A.L.Capriotti. Anal. Chem. 2018, 90, 12230−12238

P-548 Engineering candidate sets for annotation using Extended Metabolic Models (EMMs)

PRESENTING AUTHOR: ***Soha Hassoun, TUFTS UNIVERSITY, United States***

CO-AUTHORS: ***Neda Hassanpour, Alden, Nicholas, Kyongbum Lee***

A major challenge in metabolomics analysis lies in resolving the chemical identities of detected features. Computational annotation tools recommend one or more candidate chemical structure that best explains the spectral signature. These approaches often rely on a candidate set that is typically culled from a large database such as PubChem. However, not all such compounds are biologically relevant to the sample under study and there are many biologically relevant yet unknown compounds (“dark matter”) that are not catalogued at all. We propose a novel method for systematically engineering candidate datasets that can be used during annotation. Our central premise is that promiscuity of enzymes towards substrates within the sample is responsible for a large number of detected measurements. To create an EMM, a reference metabolic model, cataloged in KEGG, BIGG, or BioCyc, for the organism(s) associated with the sample, is augmented with promiscuous metabolic products. Such products are derived using PROXIMAL, a tool for predicting promiscuous products. EMM metabolites with masses that match to measurements, within a small ppm, are considered for the candidate set. We demonstrate the utility of our approach in annotation using an untargeted LC-MS dataset for Chinese hamster ovary (CHO) cells. Our test case shows that our EMMA (EMM Annotation) workflow creates a candidate set that is enzymatically relevant to the sample and that includes metabolites beyond what is already catalogued in PubChem. Further, we experimentally confirm the identification of 4-hydroxyphenyllactate, a metabolite that is currently not part of the known catalog of CHO cell metabolites.

P-549 WiPP: Workflow for improved Peak Picking

PRESENTING AUTHOR: ***Yoann Gloaguen, Berlin Institute of Health, Max Delbrück Center, Germany***

CO-AUTHORS: ***Nico Borgsmüller, Tobias Opialla, Eric Blanc, Emilie Sicard, Anne-Lise Royer, Bruno Le Bizec, Stéphanie Durand, Carole Migné, Mélanie Pétéra, Estelle Pujos-Guillot, Franck Giacomoni, Yann Guitton, Dieter Beule, Jennifer Kirwan***

High false positive rates in GC-MS metabolomics peak detection is a common issue that impedes automated analysis of large-scale data. Many algorithms are available for peak detection but performance differs depending on algorithmic approach and data acquisition method. This makes it difficult to compare between algorithms without extensive manual intervention. We present a parameter optimizing, multi-algorithm peak detection workflow for GC-MS metabolomics which automatically evaluates the detected peaks’ quality using machine learning-based classification. First, the classifier is trained to distinguish between real compound-related and false positive peaks. Then the algorithm parameters are scored based on detected peaks quality and optimized accordingly. This procedure is repeated for two peak detection algorithms and both algorithms are run in parallel on the entire data set with the optimized parameters. The qualitative information returned by the classifier for every peak is used to merge individual algorithm results into one final high-confidence peakset. Using this approach, we show that automated detection and evaluation of peak quality is possible with sensitivity of 90% on a standard compound mixture. The information generated by the classifier allows: Optimization of individual peak detection algorithms parameters to increase performances, an objective way to assess peak detection algorithm performance, optional exclusion of peaks which are of intermediate to low quality. We demonstrate that this workflow automatically recovers over 90% of the compounds identified in biological data by manual curation, so less than 10% of the peaks need user attention. The workflow is suitable for large-scale studies.

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TECHNOLOGY

P-550 A Metabolite Identification Guide for the Assignment of Unknown Signals in NMR Metabolomics

PRESENTING AUTHOR: ***Silke Heinzmann, Helmholtz Center Munich, Germany***

CO-AUTHORS: ***Philippe Schmitt-Kopplin***

Identification of molecular features is still a bottleneck in non-targeted metabolomics. Generally, only signals that are highlighted in a study in response to an intervention or associated to a (disease) phenotype are identified. However, when aiming to name and count the metabolites that are visible by NMR spectroscopy, an answer is difficult. Which and how many metabolites do we see in a given sample? Is there a set of metabolites that is present in most samples? How can we organize a straightforward workflow for the identification of metabolites? Here, we show a workflow for the identification of metabolites based on two-dimensional NMR analyses and structural correlation analyses. First, we collect as much information as possible via NMR spectroscopy by acquisition of 1- and 2-dimensional experiments. This information is then submitted to in-house and online databases (e.g. HMDB). In a second step, the CLASSY (i.e. cluster analysis statistical spectroscopy) method is used to cluster all peaks of the NMR spectrum, first locally (i.e. peaks that derive from the same molecule) and then globally (i.e. clusters that are biologically related). Thereby, known metabolites do cluster with unassigned peaks and facilitate structure elucidation by giving a suggestion on pathway relationships or chemical classes. With this approach, we have identified several signatures from host-microbial co-metabolism and food metabolites that require microbial putrefaction processes. The cluster analysis method gave valuable hints towards identification of previously not reported metabolites.

P-551 Multi-feature based data processing of Data Independent acquisition metabolomics data without retention time information

PRESENTING AUTHOR: ***Stephen Tate, SCIEX, Canada***

CO-AUTHORS: ***Pradeep Narayanaswamy, Adam Lau, Lyle Burton***

Mass spectrometry coupled with liquid chromatography is the most popular method for untargeted metabolomics that detects thousands of features from complex samples, but only a fraction of them are annotated. Each analyte can give rise to multiple features like in-source fragments (ISF), adducts, and different charge states. Chromatography retention times (RT) of the metabolites is important orthogonal information required for avoiding pitfalls due to many features having similar mass-to-charge (m/z) ratios which can interfere with known compounds. Recent advances in MS instrumentation with data-independent acquisition (DIA) mode like SWATH® acquisition gave unbiased MS/MS data capturing of all the features that are generated from metabolites. Using multiple features, we developed a scoring model for confident identification of the metabolites. LC-MS often produces multiple features along with due to adducts [Na, K, Li, NH4, Acetate, Formate, Cl etc.], multiply charged, dimers and in-source fragments. We developed a prediction model to generate in-silico MS/MS spectra for other features (ISF) based on the MS/MS of known features of M+H/M-H and then matched the observed fragments of in-silico spectra for sequence coverage against MS/MS spectra of molecular ion (M+H/M-H) to enhance the scoring of metabolites. In complex mixtures, two or more peak groups that represent product ions are located at different RT intervals along with the expected peak group. To address this challenge, we developed a model that correlates the peak groups across the SWATH windows for multiple features to identify right metabolite and reduce the false positive identifications.

P-552 Targeted metabolomics – analytical method development and validation of free fatty acids in mammal's plasma and tissue by gas chromatography mass spectrometry analysis

PRESENTING AUTHOR: ***Anna Bauer, Max Delbrück Center, Germany***

CO-AUTHORS: ***Raphaela Fritsche, Jennifer Kirwan***

Plasma free fatty acids (FFA) are lipolysis byproducts primarily derived from adipose tissue. High levels of plasma FFA have toxic effects on organs related to cardio metabolic disease. Furthermore, lifelong exposure of populations to dietary fatty acids has raised concerns about the potential links between cognitive disorders and nutrition. However, the association of FFAs with cognitive impairments or dementia is still unknown but there is obviously great interest in this field of research given the potential implications for human health. In order that this area of research can be better studied, a well validated, quantitative analytical method is required. A short and simple method by gas chromatography coupled to a mass spectrometer was developed to determine 21 FFA. The optimized methodology entailed the formation of trimethylsilyl derivates using N-Methyl-N-(trimethylsilyl) trifluoroacetamide after a one-step derivatization at 37 °C. Compared to existing methods, the aim of this method was to develop a rapid qualitative and quantitative method for individual FFA suitable for both plasma and tissues of various types. The analytical method is currently being fully validated according to European medicine guideline using external calibration curves. All standard parameters important for a well validated method have been measured and optimized including precision (intra and inter-day), stability and reproducibility, limit of detection and lower and upper limits of quantification. This is designed to work on an automated derivatization system, including the use of pooled quality control samples to both measure and reduce the technical variation in the analyses to a minimum.

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TECHNOLOGY

P-553 A complete workflow for improved untargeted metabolome annotation and identification using ultra high-resolution accurate mass and LC-MSn Orbitrap-based mass spectrometry

PRESENTING AUTHOR: ***David Peake, Thermo Fisher Scientific, United States***

CO-AUTHORS: ***Reiko Kiyonami, Ioanna Ntai, Amanda Souza, Ralf Tautenhahn***

Compound identification is the most significant bottlenecks faced by researchers in untargeted metabolomics today. The gold standard for structure elucidation in organic synthesis is a consensus of information from multiple analytical methods (2D-NMR, FTIR, UV-VIS and other methods) to characterize milligrams of purified material. For identification of nanogram levels of metabolites in human plasma using mass spectrometric methods, confident annotation of known metabolites requires very high resolution, accurate mass measurement coupled with LC-MS and MS/MS analysis. SRM1950 (NIST) human pooled plasma extract was separated on a reversed-phase C18 column and LC-MS analyses were performed using an Orbitrap mass spectrometer. Mass spectral data were acquired using 120K resolution MS and data dependent MS2 at 30K resolution. Identifications were confirmed by spiking standards into the plasma extract and neat standard run under the same conditions. Orbitrap-based mass analyzers offer many analytical advantages including ultra-high resolution, very high mass accuracy and precision, and multiple methods of ion activation with MSn capabilities for structure elucidation. By combining accurate mass measurement, isotopic pattern, fine isotope structure and high quality mass spectra including fragmentation data with multiple structure database and mass spectral library searches one can achieve high confidence in metabolite annotations. These concepts are illustrated with the analysis of NIST SRM1950 human plasma. Using an automated and flexible data processing pipeline, it is possible to obtain consensus between all data sources giving higher confidence in metabolite annotation. Ultimately, proof of identification requires comparison of a reference standard with the analyte under the same analytical conditions.

P-554 Detecting Low Abundant Endogenous Cardiac Steroids from Biological Fluids Using a Structure-Based MSn Approach on an Orbitrap Tribrid MS

PRESENTING AUTHOR: ***Sally Webb, Thermo Fisher Scientific, United States***

CO-AUTHORS: ***Reiko Kiyonami, Michael G. Harrington, Alfred N. Fonteh, Roger Biringer, Andreas Huhmer***

Endogenous cardiac steroids are specific inhibitors of the sodium pump (Na+/K+-ATPase) and play important biological roles such as regulating cell growth, differentiation, apoptosis, fibrosis, immunity, carbohydrate metabolism, and nervous and mental functions. Detecting endogenous cardiac steroids from biological fluids is very challenging because of their low concentration (ng/ml) ranges and lack of authentic standards. We have developed a novel structure-based MSn discovery approach which enables rapid annotation of cardiac steroid class compounds by identifying basic steroid sub-structure shared by cardiac steroid class compounds using MS2 and MS3 spectral tree data. We applied this approach to cerebrospinal fluid (CSF) samples and were able to detect some endogenous cardiac steroids using complementary information of MS2 and MS3 data. A Thermo Scientific™ Orbitrap ID-X™ Tribrid™ mass spectrometer was used and MSn data was collected using an MS2 data directed data dependent approach. Thermo Scientific™ Mass Frontier™ 8.0 software and Thermo Scientific™ Compound Discoverer™ 3.0 software was used for data processing with mzCloud™ spectral library and ChemSpiderTM database employed for the structural annotation of detected cardiac steroid class compounds. Three cardiac steroid-like compounds were detected from a CSF sample using the structure-based approach. Although the signals of these steroid class compounds are relatively low, both MS2 and MS3 data were being collected on these compounds, improving the sensitivity and selectivity of our workflow. A targeted PRM assay of three detected putative cardiac steroids was used to multiple CSF samples for verifying they could be detected reproducibly from other CSF samples.

P-555 Innovations in Unknown Identification for Metabolomics and Lipidomics

PRESENTING AUTHOR: ***Richard Yost, University of Florida, United States***

CO-AUTHORS: ***Robin H.J. Kemperman, Jeremy P. Koelmel, Allison J. Levy, Russell Lewis, Nicholas R. Oranzi, Jiajun Lei, Matthew E. Merritt, Timothy J. Garrett***

NIH has funded a new consortium charged with “catalyzing the field of compound identification to dramatically increase the number of identified biomedically-relevant metabolites in untargeted metabolomics”. This presentation will explore innovations in identifying unknown metabolites and lipids; the primary focus will be on innovations at UF, but will include a perspective on innovations from the twelve other centers within the consortium. Innovations for identifying unknown metabolites and lipids include two promising strategies, ion mobility and LC/MS/MS/NMR. Ion mobility/mass spectrometry can separate isomeric metabolites, lipids (including eicosanoids and steroids) that are not resolved by chromatography and not readily differentiated by MS/MS. Furthermore, determining an unknown’s collision cross section can help determine its structure. We are developing software for processing LC/MS/MS data (including ion mobility data) that provides a wide coverage of lipids and improved lipid annotations. Integrating LC/MS/MS with microflow NMR could provide the ideal platform for identification of unknown metabolites and lipids. Although MS/MS is more sensitive and functions on the chromatographic timescale, the structural information provided by 1H NMR is invaluable, despite its lower sensitivity and longer timescale. By digitizing the LC effluent using segmented flow, the timescale of LC and NMR is decoupled and “smart” selection of LC segments (based on MS/MS data) allows targeted NMR analysis. Initial experiments are focused on differentiating lipid isomers, with extension to structure elucidation of unknown metabolites. Experimental details and recent application of these strategies will be highlighted, along with a perspective on their impact in untargeted metabolomics and lipidomics.

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*AWARD WINNERS

TECHNOLOGY

P-556 Combining Mass Difference Networks with Spectral Meta Data Analysis for Structural Characterization

PRESENTING AUTHOR: ***Kris Morreel, Plant Systems Biology, VIB, Belgium***

CO-AUTHORS: ***Sandrien Desmet, Atsushi Fukushima, Rebecca Dauwe, Geert Goeminne, Yvan Saeys, Wout Boerjan***

Mass difference networks, such as Candidate Substrate Product Pair (CSPP) networks, implement knowledge on biochemical conversions for the structural characterization of unknowns. This typically occurs via network propagation in which nodes and edges represent m/z features and enzymatic conversions. Including Collision-Induced Dissociation (CID)-spectral similarities enhances both the efficiency and the reliability of this approach. Here, we present RDynLib, an R package that (i) associates different types of CID spectra, i.e. MS/MS and MSn, to the CSPP network, and (ii) provides a set of functions to improve the elucidation of especially the unknown unknowns via a combined CSPP / CID spectral meta data analysis. RDynLib is based on the alignment of chromatograms from different MS instruments following the prior archiving of all recorded CID spectra into the DynLib database. The benefit of a CID spectral meta data analysis is demonstrated by matching the CID spectra against several freely available CID spectral databases.

P-557 Assessment of in silico NMR chemical shift library errors and a new method to automate explicit and implicit solvent conditions

PRESENTING AUTHOR: ***Yasemin Yesiltepe, Washington State University, United States***

CO-AUTHORS: ***Sean Colby, Jamie Nunez, Niranjan Govind, Mark Borkum, Nancy Washton, John Cort, Thomas O. Metz, Justin Teeguarden, Ryan Renslow***

The majority of metabolites are not available as authentic reference material, making confident identification of most molecules in complex samples unachievable. Also, the cost of synthesizing/purchasing standards and collecting experimental data on each individually, represents a major roadblock. A promising approach to expand publicly available databases is through in silico calculation of molecular attributes. We developed the in silico Chemical Library Engine (ISiCLE), a Python-based pipeline, designed to calculate chemical properties, including nuclear magnetic resonance (NMR) chemical shifts, by employing density functional theory (DFT). Using ISiCLE, we investigated the accuracy required for identification of 11,000 chloroform and water-soluble metabolites found in the Human Metabolome Database (HMDB). Of these, 90% can be correctly identified in a pure sample when errors of 1H and 13C chemical shifts reach at least 0.7 ppm and 6 ppm, respectively. Furthermore, to decrease the calculated chemical shift error, it is possible to consider explicit solvation of metabolites. This is especially critical for polar molecules, due to their strong interaction with polar solvents. We built an automated solvation module of ISiCLE to evaluate explicit and implicit solvation at varying levels of theory. This method generates a large solvation box, filled with a solvent of interest, surrounding the analyte, and subsequently optimizes the whole system. Then, an optimum finite number of solvent shells is determined, and the remaining system is simulated with an implicit solvation model (i.e., COSMO). We anticipate this automated solvation pipeline will enable efficient and rapid identification and delineation of metabolites in custom solvent conditions.

P-558 Automated Screening of GC×GC-TOFMS Metabolomics Data Using Scripting

PRESENTING AUTHOR: ***Seo Lin Nam, University of Alberta, Canada***

CO-AUTHORS: ***Seo Lin Nam, A. Paulina de la Mata, James Harynuk***

Comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry is an analytical platform with high separation efficiency and is a powerful tool for the hybrid target/non-target analysis of complex mixtures. However, the amount of data generated from such comprehensive techniques is massive and nearly impossible to handle manually. The difficulty and complexity of data analysis is the major bottleneck in GC×GC-TOFMS analysis, rather than the usual culprits of sample preparation or instrumental time. The challenge of data processing is holding GC×GC-TOFMS back from more widespread use. In order to speed up and simplify data analysis, script-based filtering of peaks is a promising tool. Scripting is a programming language of logic rules using mass spectrometric and/or retention properties for target compounds such as fragmentation patterns and/or the presence/absence of specific ions. The use of scripting can help to quickly find and visualize compounds of interest and particular chemical classes in a sample of thousands of peaks. For environmental and petroleum work, there are numerous scripts that have been published to aid with, for example, the identification of halogenated species. Presented herein is the recent progress towards the development of a suite of scripts that classifying peaks in GC×GC-TOFMS data from metabolomics studies. This includes the identification of fatty acid methyl esters, free fatty acids, aldehydes, alcohols, amino acids, ketones, sterols and sugars.

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TECHNOLOGY

P-559 Automated annotation of untargeted LC-MS metabolomics data using all-ion LC-MS/MS and in-silico fragmentation information

PRESENTING AUTHOR: ***Gonçalo Graça, Imperial College London, United Kingdom***

CO-AUTHORS: ***Yuheng Cai, ChungHo Lau, Panagiotis Vorkas, Elizabeth J. Want, David Herrington, Timothy M. D. Ebbels***

Untargeted metabolomics LC-MS experiments produce complex datasets containing tens to hundreds of thousands of features (m/z_retention time pairs) from thousands of molecules. The annotation of such features is a bottleneck, even for known compounds, requiring additional MS/MS experiments and expert knowledge of molecular fragmentation [1]. In all-ion LC-MS/MS schemes such as alternated low and high collision energy acquisitions (MSe or MSall) and sequential window acquisition of all theoretical mass spectra (SWATH) provide MS fragmentation data at no additional experimental time cost [2]. However, reconstruction of parent-fragment relationships is a difficult task, particularly for wide fragmentation windows experiments (MSe or MSall) which generate spectra composed of fragments from multiple parent ions. In the presented work we propose a novel approach for automated annotation of LC-MS using all-ion fragmentation data, which combines correlation-based parent-fragment ion reconstruction with in-silico molecular fragmentation and retention-time information. This strategy allowed the correct annotation of 155 of 207 features (75%) from XCMS outputs [3] in a set of human serum samples. These corresponded to different isotopologues, adducts and fragments from lipid and other small metabolites. This approach provides a useful framework for the annotation and interpretation of untargeted LC-MS metabolomics data and can be easily adapted to other samples and experimental settings. An R package, including dedicated visualisation tools, is planned to be released in the near future. References: [1] Domingo-Almenara et al. Anal. Chem. (2018), 90, 480−489. [2] Broeckling et al. Anal. Chem. (2014), 86, 6812−6817. [3] Smith et al. Anal. Chem. (2006), 78, 779–787.

P-560 Facilitating interpretation of metabolomics data using KEGG and HMDB databases

PRESENTING AUTHOR: ***Cédric Bovet, Bern University Hospital, Switzerland***

CO-AUTHORS: ***Zhaoyue He, Stefan Zahnd, Katrin Freiburghaus, Carlo R. Largiadèr, Martin G. Fiedler***

The annotation of metabolic ions and their assignment to pathways remains the main challenge in non-targeted metabolomics by high-resolution mass spectrometry (HRMS). To gain annotation confidence, laboratories are often measuring hundreds of metabolite standards and generate in-house spectral libraries. Usually, only a small proportion of the detected ions is annotated with this strategy. To enhance the annotation of unknown ions, we developed R and Python packages to extract the most probable metabolites and chemical taxonomy (i.e., class, sub-class, etc.) from HMDB and KEGG databases based on measured m/z ratios. After tentative annotation of ions and pathways using Mummichog (http://mummichog.org/), our developed R package assigns the generated list of empirical compounds to the corresponding fold changes of the input file, downloads the corresponding KEGG maps and generates a comprehensive overview of impaired metabolic pathways. Potentially identified metabolites are shown as nodes colored and sized according to their fold change and p-value, respectively. The Python package extracts the potential metabolite identities for each measured m/z ratio and the chemical taxonomy from HMDB. An annotation tolerance for the taxonomy of interest can be specified and the filtrated annotation is used to reveal collectively regulated classes. To test our approach, we reanalyzed a dataset obtained from male adults with type-1-diabetes undergoing exercises [1] and successfully annotated unknown ions and isolated regulated pathways (i.e., purines, steroids and amino acids). In conclusion, our approach facilitates the annotation of ions and the interpretation of complex non-targeted HRMS data. [1] L. Bally et al. Metabolomics, 13, 78.

P-561 A novel UHPLC-MS method for global profiling of cholesteryl esters and potential biomarkers discovery of hyperlipidemic golden hamsters

PRESENTING AUTHOR: ***Miao Lin, Institute of Materia Medica, CAMS & PUMC, China***

CO-AUTHORS: ***Zhe Wang, Dongmei Wang, Xiong Chen, Jin-Lan Zhang***

Cholesteryl esters (CEs) play crucial roles in cholesterol homeostasis, and abnormal levels of CEs result in various diseases, such as Wolman disease, hyperlipidemia, atherosclerosis, and cancer. Global profiling of CEs in biosamples would be beneficial to the discovery of disease biomarkers. In our study, we reported the global profiling of CEs in plasma and three different lipoproteins (VLDL, LDL, and HDL) of golden hamsters by a mathematical model-assisted ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS) method. Seventy-four CEs (50 newly reported CEs) were identified in golden hamster with the validated mathematical strategy based on the chromatographic retention behavior, structural features and CE standards. Then, 74 CEs were quantified by dynamic multiple reaction monitoring (dMRM) for the discovery of potential biomarkers in hyperlipidemic golden hamsters. A total of 57, 52, 42, and 41 CEs were indicated as potential biomarkers in the plasma, VLDL, LDL, and HDL of hyperlipidemic golden hamsters, respectively. We found that 24 CEs were shared in plasma and lipoproteins. And 11 CEs were reported to be closely related to metabolic disorders and heart diseases, while 13 CEs were novel potential biomarkers of hyperlipidemia. Our study expands the scope of CE compound analysis in biosamples and can be applied for the discovery of biomarkers for human diseases.

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*AWARD WINNERS

TECHNOLOGY

P-562 Metabolomics analysis in rat plasma during short-term abstinence following methamphetamine self-administration

PRESENTING AUTHOR: ***Suji Kim, College of Pharmacy, Keimyung University, South Korea***

CO-AUTHORS: ***Suji Kim, Hyerim Yu, In Soo Ryu, Sooyeun Lee, Sangkil Lee***

Drug addiction is a chronic, relapsing brain disease that is strengthened by a dynamic process of repeating drug reward and withdrawal. Drug withdrawal, usually starting within hours of the last dose, is a critical step in the transition from reward to addiction. Methamphetamine (MA) is a highly addictive central nervous system stimulant and its abuse has become a serious global public health concern. In the present study, a time-series metabolomics analysis was performed to uncover metabolic changes associated with MA short-term abstinence. Metabolic alterations were investigated in rat plasma collected immediately after 16 d of MA self-administration (SA) and after 12 and 24 h of abstinence using both non-targeted UPLC-QTOF-ESI-MS and targeted MS of amino acids, biogenic amines, acylcarnitines, glycerophospholipids, and sphingolipids. Principal component analysis revealed that the highest level of separation occurred between the 24 h and control groups from the UPLC-QTOF-ESI-MS data. We detected 358/445/453 and 521/618/584 significantly different ion features in the SA/12 h/24 h groups in positive and negative ESI, respectively, including 5-methylcytosine, deoxycytidine, glycocholate, nicotinamide, corticosterone, hydroxyisobutyric acid, linolenic acid, hippurate, 3-(4-hydroxyphenyl) lactate, taurine, urate, and uridine. We also found that, during the short-term abstinence from MA self-administration, the levels of many amino acids, biogenic amines, and glycerophospholipids were significantly altered. Specifically, distinct changes were observed in the metabolic pathways involved in energy metabolism, the nervous system, and membrane lipid metabolism. These findings provide essential knowledge of the dynamic metabolic effects associated with short-term MA abstinence and may help identify early warning signs of MA dependence.

P-563 Improved Metabolite Identification in a Single Injection with SWATH Acquisition for Untargeted Metabolomics Workflow

PRESENTING AUTHOR: ***Robert Proos, SCIEX, United States***

Comprehensive metabolite identification with MS/MS library spectral matching can be problematic for data dependent acquisition (DDA) workflows as it often requires multiple injections for each sample. SWATH Acquisition, a Data Independent Acquisition (DIA) method, with optimized variable windows, provides a powerful workflow requiring only a single injection per sample for each polarity. In addition to capturing product ion spectra for all ionizing analytes, SWATH also provides the option of quantitation at either the MS or MS/MS level allowing a comprehensive qualitative and quantitative analysis of metabolites in complex biological samples like plasma.

P-564 The GERSTEL Metabolomics Prepstation

PRESENTING AUTHOR: ***Nathan Hawkins, GERSTEL GmbH & Co.KG, Germany***

Robust, reproducible sample preparation methods are fundamental to the delivery of high-quality metabolomics and metabolic phenotyping datasets. Whilst the last decade has seen great improvements in instrumental precision, sensitivity and robustness, most sample preparation is still done manually, and is a significant bottleneck to sample throughput and the principal source of experimental error. Whilst some labs have automated sample preparation to reduce human error and improve analytical precision, most laboratory robotics systems are either dedicated liquid handling systems or expensive bespoke systems designed and programmed to automate a single protocol in batches. Dedicated liquid handling robots have limited sample preparation capability (cannot automate evaporation, vortexing, centrifugation and solid phase extraction) and bespoke systems lack the flexibility to modify the protocol to deal with different samples/matrices to meet changing laboratory needs or improve methods to meet quality or regulatory requirements. Furthermore, whilst preparing samples in batches works for molecular biology and proteomics, metabolites (or their derivatives) that are not stable are excluded during data pre-processing prior to statistical analysis. We have therefore developed a flexible, modular, user-configurable and programmable Prepstation that can fully automate the most commonly used LC/LC-MS, GC/GC-MS and NMR protocols for lipidomics, metabolomics and metabolic phenotyping including: The Fiehn Protocol (MOX-TMS).Biomedical research into inborn errors or metabolism (tBDMS). Amino Acid analysis as their Alkyl Chloroformates.Metabolic phenotyping of microbial, plant, and animal FAMEs. The GERSTEL Metabolomics Prepstation can be configured for a wide range of metabolomics and lipidomics protocols including those for commercially available kits.

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TECHNOLOGY

P-565 Untargeted metabolomics application to monitor urine samples by ultra-high-pressure liquid chromatography coupled to high-resolution mass spectrometry

PRESENTING AUTHOR: *Csaba Laszlo, Philip Morris International, Switzerland*

CO-AUTHORS: *A. Haiduc, E. Dossin, F. de Jong, C. Beecher, A. Kamleh, P.A. Guy, N.V. Ivanov, M. Peitsch*

In order to meet the demands of high-throughput metabolomics analysis, we established urine sample preparation method on 96-well plates and an efficient liquid chromatography (LC) coupled to high-resolution mass spectrometry (MS) method using the latest model of the Vanquish™ Tandem LC system. The separation of urine metabolites was performed using two Hypersil GOLD™ C18 columns (150 x 2.1 mm, 1.9 µm) operating in parallel and running with a fast linear gradient of acetonitrile containing 0.1% formic acid, ramping from 5% to 95% in 10 minutes, alternating between the two columns. Dual-column operation with column reconditioning maintains full occupancy of the MS instrument (MS acquisition of column A during re-equilibration of column B). MS detection was realized on a Q Exactive™ HF mass spectrometer operating in positive and negative electrospray ionization acquisition modes. Data quality consistency was assessed through various pooled urine quality control samples injected on both columns processed by principal component analysis. In order to prevent possible drift in retention time across the columns, various reference index markers eluting across the gradient were analyzed at the beginning and the end of the 96-well plate series on each column. Retention index values were calculated to provide reproducible results over time. In addition, we have assessed the 13C yeast extract (TruQuant IQQ Workflow Kit, IROA®) to obtain a more robust method for metabolite identification and quantification.

P-566 Electromembrane extraction for the analysis of betaine, carnitine, choline, deoxycarnitine and TMAO in plasma

PRESENTING AUTHOR: *Drouin Nicolas, Leiden University, Netherlands*

CO-AUTHORS: *Amy Harms, Tim Kloots, Julie Schappler, Serge Rudaz, Isabelle Kohler, Petrus Wilhelmus Lindenburg, Thomas Hankemeier*

Choline, trimethylamine N-oxide (TMAO), carnitine, deoxycarnitine and betaine are now routinely quantified as potential biomarkers of cardiovascular disease. The common analytical workflow typically relies on protein precipitation and flow injection combined with the use of deuterated internal standards for high-throughput analysis. However, this approach leads to significant matrix effects and up to a 80% sensitivity decrease due to the presence of salts and phospholipids. In this context, electromembrane extraction (EME) appears to be a promising alternative to selectively extract polar ionizable compounds from complex matrices such as plasma. In this study, EMEs were performed in parallel using an in house Parallel-EME device and experimental parameters were optimized. Nitrophenyl pentyl ether (NPPE) was selected as supported liquid membrane based on the physico-chemical properties of the targeted compounds. Intrinsic parameters such as voltage and or applied current evaluated as well as sample composition (i.e., simple plasma dilution, MeOH addition, and protein precipitation) were. Optimal conditions, namely 400 µA/well, 1400 rpm, 15 min, protein precipitation using trichloroacetic acid to reduce the ionic strength and the buffer capacity of plasma, lead to repeatable extraction as the observed process efficiency as lower than 20%, and further improved to <5% using internal standard correction. The developped sample preparation method allowed to reduce the matrix effect up to a 2-fold factor, leading to a sensitivity improvement of 3-fold for compounds such as TMAO. In addition, this new approach was successfully applied to a cohort of 40 plasma samples, demonstrating the potential of Pa-EME in targeted metabolomics.

P-567 Development of a neurochemical profiling method in rat plasma using liquid chromatography-tandem mass spectrometry

PRESENTING AUTHOR: *seungju kim, Keimyung University, South Korea*

CO-AUTHORS: *Byoungduck Park, Sooyeun Lee*

Tyrosine and tryptophan are precursors of major neurotransmitters including dopamine and serotonin, which are key in modulating brain functions and neurological diseases. Comprehensive measurement of not only neurotransmitters but also their precursors and metabolites is thus essential to understand the relationship between the pathophysiological states and the neurological actions. However, accurate measurement of those neurochemicals remains challenging due to very low basal concentrations and diverse chemical properties (e.g. polarity). In the present study, a metabolic profiling method for 16 neurochemicals derived from tyrosine and tryptophan was developed and validated in rat plasma using liquid chromatography-tandem mass spectrometry combined with chemical derivatization. The validation results proved the method to be selective, sensitive, accurate and precise, with acceptable linearity within calibration ranges. The lower limits of quantification ranged from 0.05 to 50 ng/mL. This is a powerful neurochemical profiling method for monitoring the metabolic pathways of dopamine, norepinephrine, serotonin and kynurenine in rat plasma and will be very useful to further studies on the regulation of the neurotransmitter metabolic pathways in vivo.

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*AWARD WINNERS

TECHNOLOGY

P-568 Combination of UHPLC coupled to Q-TOF and QQQ provides substantial benefits for metabolic pathway profiling

PRESENTING AUTHOR: ***Katrin Freiburghaus, University Institute of Clinical Chemistry, Switzerland***

CO-AUTHORS: ***Carlo Largiadèr, Christoph Stettler, Martin G. Fiedler, Lia Bally, Cédric Bovet***

Non-targeted metabolomics by UHPLC coupled to high-resolution mass spectrometry (HRMS) is often applied for identification of regulated pathways in biological samples. Although this approach facilitates the discovery of new metabolites, it suffers from detector saturation and a complex integration pipeline. The simple data integration and scheduling acquisition has fostered the use of triple quadrupoles (QQQ) for profiling hundreds of metabolites in a single run. Here, we demonstrate the benefits of a joint use of non-targeted HRMS and targeted QQQ metabolic profiling to characterize exercise-induced metabolic changes in serum of type-1-diabetes (T1D) patients. In a crossover study, 12 male adults with T1D underwent 90 min of intermittent high-intensity or iso-energetic continuous moderate-intensity exercise. Metabolic profiling of serum collected before, during and after exercise was performed by UHPLC-HRMS (Q-TOF) and QQQ. The QQQ method covered 114 metabolites of which 97 were detected in the samples. Amongst the 83 commonly detected metabolites, intra-assay precision was increased up to 2-fold using QQQ compared to HRMS. Up to 61% of the detected metabolites had an excellent linearity over three orders of magnitude (r2 >0.99) with the QQQ, while only 29% with the HRMS. Selected pathway coverage was improved up to 3-fold by the QQQ: 62% metabolite coverage of purine metabolism and 33% coverage of citric acid cycle were achieved. Our screening strategy enhanced metabolic profiling in serum of T1D patients by combining the benefits of unbiased full-scan HRMS acquisition with extended insights into specific pathways by QQQ analysis.

P-569 Micro-LC-MS versus UHPLC-MS analysis of endocannabinoids in cerebrospinal fluid: the potential role of the endocannabinoid system in migraine pathology

PRESENTING AUTHOR: ***Xinyu Di, LACDR, Netherlands***

CO-AUTHORS: ***Faisa Guled, Elke Krekels, Isabelle Kohler, Thomas Hankemeier.***

Experimental and clinical data have suggested a link between migraine pathology and the endocannabinoid system (ECS). In this study, we aim to compare endocannabinoid levels in cerebrospinal fluid (CSF) and plasma samples in migraine patients (N = 195) versus healthy controls (N = 94). The problem here is, the concentration of several endocannabinoids in CSF is very low (sub-ng/mL range), lower than the limits of detection (LODs) offered by conventional UHPLC-MS techniques, which highlights the need for highly sensitive detection methods. To achieve this goal, a micro-LC-MS method has been developed and optimized for the targeted analysis of 17 endocannabinoids. The separation was performed using a micro Acquity HSS T3 column (0.3 × 100 mm, 1.8 µm) at a flow rate of 10 µL/min. An injection volume of 4uL is applied by utilizing the enrichment function of the column. Electrospray ionization and selective Multiple Reaction Mode was used for data acquisition. The lower limits of quantification (LLOQs) obtained with the developed micro-LC-MS method were 5 to 10 times lower than the LLOQs obtained with state-of-the-art UHPLC-MS method using the same mass analyzer. The developed method enabled the detection of the 17 targeted endocannabinoids in human CSF. This method will be applied to the analysis of migraine patient samples.

P-570 Balancing quantitative and qualitative LC-HRMS for simultaneous targeted and non-targeted metabolomics

PRESENTING AUTHOR: ***Anne-Charlotte Dubbelman, Leiden University, Netherlands***

CO-AUTHORS: ***Filip Cuyckens, Lieve Dillen, Rob J. Vreeken, Thomas Hankemeier***

A typical trend in metabolomics is to do more for less. In mass spectrometry (MS), this induces the shift from using triple quadrupole MS for targeted and more quantitative metabolomics versus high-resolution MS (HRMS) for non-targeted metabolomics, towards using HRMS for both at the same time (Quan/Qual). Although theoretically attractive, practically, optimizing HRMS parameter settings for qualitative analysis can compromise the quantitative analysis and vice versa. Therefore, we investigated the often underestimated effect of selected HRMS parameter settings on the quantitative and qualitative performance. Human plasma was used for all evaluations and a wide variety of drugs was spiked to optimize for (pharmaco-)metabolomics. On ultra-high performance liquid chromatography (UHPLC) coupled to quadrupole Time-of-Flight MS systems, we varied and evaluated the effects of varying the scan protocol, mass resolution, scan time and smoothing on the measurements. It was found that especially for high-throughput UHPLC, the scan time (cycle time) is critical in Quan/Qual analysis. A longer cycle time allows more elaborated qualitative scan protocols, e.g. including fragmentation without or after ion mobility separation, apart from just ToF-MS. However, a too long scan time (here already observed from 200 ms) resulted in the co-detection of chromatographically separated isomers. Using a Synapt G2S MS, the amount of qualitative information correlated negatively with the quantitative performance (precision, accuracy, linear dynamic range, sensitivity). To conclude, optimizing HRMS settings for simultaneous targeted and nontargeted metabolomics requires balancing parameter settings for quantitative and qualitative performance. This study resulted in recommendations to help future Quan/Qual LC-HRMS method development.

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TECHNOLOGY

P-571 A REAL TIME METABOLOMICS PROFILING APPROACH USING RAPID EVAPORATIVE IONIZATION MASS SPECTROMETRY (REIMS) TO CLASSIFY MEAT SAMPLES

PRESENTING AUTHOR: *Yann Guitton, Laberca, France*

CO-AUTHORS: *G. DERVILLY-PINEL, S. STEAD, B. LE BIZEC*

In recent years the development of Ambient Mass Spectrometry (AMS) techniques has boomed. AMS technics are convenient when it comes to high-throughput and complex samples analysis, as it allows the analyst to bypass long sample preparation steps and gets “instantaneous” results. Among them, Rapid Evaporative Ionization Mass Spectrometry (REIMS) is promising for classification of tissue samples. In short, samples are simply “burned” and the created aerosol is ionised and directly analysed in full scan mode. On tissue samples, the REIMS generated mass spectrometric profiles are dominated by lipids. That specificity is used to discriminate samples by their lipidic fingerprint. The combination of REIMS with untargeted metabolomics workflow was investigated to identify carcasses from pig treated animals on the basis of a modification of indirect metabolites profile due to the use of Ractopamine, a β-agonist substance that may be used in some parts of the world as growth promoter in livestock, although forbidden in a number of countries. The strategy was found successful when tested on different muscle types (loin, shoulder and ham). Multi-variate statistical software package LiveIDTM (Waters) was used as a model builder and direct recognition tool. Classification performances were 0% false negative and 10 % false positive, which fully answers requirements of a screening strategy. REIMS implemented in an untargeted-metabolomics workflow can be considered as a high-throughput and powerful strategy for real-time meat classification in relation to Ractopamine treatment in pig. [1] Guitton et al DOI: 10.1080/19440049.2017.1421778 *: Corresponding author: e-mail: laberca@oniris-nantes.fr

P-572 Standardization and Quantitative Analysis in Targeted Metabolomics

PRESENTING AUTHOR: *Therese Koal, Biocrates Life Sciences AG, Austria*

CO-AUTHORS: *Hai Pham Tuan, Doreen Kirchberg, Barbara Wolf*

Quantitative metabolite analysis of related biochemical pathways is of high interest for a better understanding of health and disease. Mass spectrometry is a key analytical technique presently used in metabolomics. Reliable analytical results and improved inter-laboratory comparability, automation, and standardization of a metabolomics workflow is of utmost importance to deliver translational data for next-gen metabolomics. Here, we present the standardized, quantitative AbsoluteIDQ® p180 kit-assay as a solution for metabolic phenotyping on an Agilent triple quadrupole LC/MS system allowing the multiplexed and targeted analysis of up to 188 endogenous metabolites and lipids from six different key metabolite classes (21 amino acids, 21 biogenic amines, 40 acylcarnitines, hexoses, 15 sphingolipids, and 90 glycerophospholipids). The AbsoluteIDQ® p180 kit is applicable to a broad range of biological matrices (blood, tissue, cell culture etc.) and species and requires only 10 µL of sample volume. An easy-to-use and rapid sample preparation protocol with a specially designed 96-well filter plate allows high-throughput analysis. The LC-MS/MS instrument consists of the 1290 Infinity II LC system coupled to a 6470 triple quadrupole mass spectrometer. Automated data analysis of >22K MRM chromatograms from >230 metabolites and internal standards analyzed in 96 tests (blanks, QC, standards, and samples) was performed using Biocrates’ MetIDQ™ software, which automatically controls the entire workflow, from sample registration to data processing and result reporting. Analytical performance, including lower and upper limits of quantitation, intra- and inter-batch accuracy, and precision will be presented. In addition, a comparative study of common biofluids relevant for metabolomics will be shown.

P-573 MxP® Quant 500 Kit – Novel Standardized Metabolomics/Lipidomics Analysis Tool for Comprehensive Targeted Profiling

PRESENTING AUTHOR: *Ulf Sommer, Biocrates Life Sciences AG, Austria*

CO-AUTHORS: *Hai Pham Tuan, Svenja Heischmann, Doreen Kirchberg, Xenia Iwanowa, Radu Talmazan, Barbara Wolf, Martin Buratti, Rosa Argamasilla Martinez, Cornelia Röhring, Therese Koal*

For comprehensive metabolomics/lipidomics analysis, analytical reliability, inter-laboratory comparability, automation, and standardization are of utmost importance. Here, we present the newly developed quantitative MxP® Quant 500 kit-based assay for multiplexed MS/MS analysis of 630 metabolites/lipids from 26 analyte classes in only 10 µL sample volume. The assay allows standardized analysis in a variety of biological sample matrices (e.g. blood, feces, tissue) and species, including the microbiome. The ready-to-use assay combines UHPLC- and FIA-MS/MS into a single workflow. Automated data analysis of analytes and internal standards analyzed in 96-well format (blanks, QCs, standards, and samples) was performed with Biocrates’ MetIDQ™ software, which controls the entire workflow from sample registration to data processing and reporting. For beta-testing 14 samples (NIST SRM 1950, 6 human plasma samples, 2 human serum samples, lipemic human plasma, mouse plasma, rat plasma, mouse liver, and human feces) were evaluated. The assay was successfully validated following EMA/FDA guidelines. Absolute quantitation in biological samples was achieved using a seven-point calibration or a one-point calibration with stable-isotope labeled internal standards. The analytical accuracy and precision of the assay were safeguarded by three levels of QC samples. We will present the analytical workflow and performance parameters, including intra/inter-batch accuracies and precision, and accuracy data of reference materials. MetIDQ™ software enabled data processing including quality assessment and the combination of all results. Beta-test data across 8 independent data sets across the 14 samples will be presented showing excellent accuracies and precision results, the basis for longitudinal robustness and inter-laboratory comparability.

POSTER SESSIONS 1 AND 2 – Monday and Tuesday – all odd number posters will be on display.
POSTER SESSIONS 3 AND 4 – Wednesday and Thursday – all even number posters will be on display.

*AWARD WINNERS

TECHNOLOGY

P-574 A New HILIC LC/Q-TOF Method for Metabolomics Analysis with Important Isomer Separation and Broad Coverage of Metabolite Classes

PRESENTING AUTHOR: *Yuqin Dai, Agilent Technologies, United States*

CO-AUTHORS: *Jordy J. Hsiao*

A robust and high performance HILIC LC/Q-TOF method was developed for the detection of broad classes of endogenous metabolites. The chromatographic gradient, mobile phase pH, buffer concentration, and column temperature were optimized to achieve a baseline separation for 10 pairs of biologically important isomers. Moreover, the effect of sample salt concentration on the reproducibility of retention time (RT) and signal response was investigated. The preliminary results showed that RT and signal response reproducibility was excellent in the presence of 80 mM of NaCl and 160 mM urea. Phosphorylated metabolites such as sugar phosphates and nucleotides are often challenging to analyze due to their interactions with stainless steel in the system. By incorporating medronic acid to deactivate the steel surfaces, CV values were within 0.2% and 10% for RT and signal response respectively. The effect of source conditions on MS response was also evaluated. The results showed that gas temperature and nozzle voltage play a key role in altering the signal response. Low temperature favored labile organic acids, while high temperature enhanced phosphorylated metabolites. By using optimal source conditions, this method enabled the detection of broad classes of metabolites, including amino acids, vitamins, polyamines, carboxylic acids, sugars and sugar phosphates, nucleotides, cofactors and coenzyme A (CoA) derivatives. This new HILIC LC/Q-TOF method clearly demonstrated a superior analytical performance in terms of the chromatographic separation, analytical reproducibility, and global coverage of metabolites. Implementation of this method for the analysis of real-world biological samples is being conducted.

P-575 High-throughput lipidomics and metabolomics analysis directly on genetically modified CHO cells using Laser-Ablation Rapid Evaporative Mass Spectrometry (LA-REIMS)

PRESENTING AUTHOR: *Stefania Maneta-Stavrakaki, Department of Surgery and Cancer, Imperial College London, United Kingdom*

CO-AUTHORS: *Julia Abda, Alvaro Perdones-Montero, Simon J. Cameron, Yuen-Ting Chim, Paloma Diaz-Fernandez, Zoltán Takáts*

Introduction - Chinese Hamster Ovary (CHO) cells are used as host cells for the production of recombinant proteins. The analysis of CHO cells is difficult , due to the time-consuming extraction methods, which can create a bias regarding the cells' biological profile. Here, we report a high-throughput lipidomic and metabolomic analysis in ambient conditions, with minimal sample treatment, using Laser-Ablation Rapid Evaporative Ionization Mass Spectrometry (LA-REIMS) that allows the cell analysis in less than 10 seconds per sample. Methods - LA-REIMS was performed on the cell pellets of five CHO-K1 genetically modified cell lines – producing different amounts of the recombinant protein adalimumab – using an OPO (optical parametric oscillator) and a CO2 laser, connected through a REIMS source interface to a QToF mass analyser. The CO2 laser offers automated analysis, since it is implemented on a TECAN platform, which allows the analysis of hundreds of samples per day. Results - LA-REIMS differentiated the five cell lines and provided many m/z values that could be of biological importance regarding the recombinant protein expression and proved to be a suitable technique for the fast analysis of cell pellets in ambient environment with minimal sample pretreatment. The next steps of the current study include MS/MS analysis on the statistically significant m/z values. Conclusion - LA-REIMS was used for high-throughput analysis of CHO cells with minimal pre-treatment. It is a fast method that can provide a biological snapshot of cells in ambient conditions.

P-576 Reference-free metabolite identification by infrared ion spectroscopy

PRESENTING AUTHOR: *Kas Houthuijs, Radboud University, Netherlands*

CO-AUTHORS: *Giel Berden, Udo Engelke, Leo Kluijtmans, David Wishart, Ron Wevers, Karlien Coene, Jos Oomens and Jonathan Martens*

A major challenge in metabolomics is identifying the full molecular structure of low-abundance small molecules. Nanomolar concentrations can be detected by combining liquid chromatography and (tandem) mass spectrometry (LC-MS), but structural information is limited to retention times and (fragment) masses, which are often inconclusive. Recently, infrared ion spectroscopy (IRIS) has emerged as a powerful analytical technique to elucidate molecular structure. IRIS combines the sensitivity of LC-MS with the structural information of gas-phase IR spectroscopy and has been successfully applied for the identification of low-abundance biomarkers. However, identification using IRIS is time consuming and relies heavily on reference compounds that are often not readily available. Here we present an automated workflow to assist in the identification of detected features in an LC-MS experiment using IRIS and computational chemistry. We are able to theoretically predict IR spectra for candidate molecules that can subsequently be scored against the experimental IR spectrum of an observed m/z feature. By linking the workflow to the human metabolome database (HMDB), the user is allowed to specify search criteria which are used to find and generate candidate structures. We benchmark the workflow by identifying metabolites using only their chemical formula and experimental IR spectra as input information. Results show that the workflow is successful in scoring the correct metabolite as the best match. Additionally, it assigns high scores to metabolites closely related in structure. The latter shows the specificity of IR and the potential the workflow has in aiding the identification of unknown unknowns.

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*AWARD WINNERS

TECHNOLOGY

P-577 How to automate boring lipidomic extraction

PRESENTING AUTHOR: ***Justine Bertrand-Michel, MetaToul-Lipidomic, France***

CO-AUTHORS: ***Julia SOULLIER, Aurélie BATUT, Jessica DALLOUX-CHIOCCIOLI, Anthony TOURNADRE, Pauline LE FAOUDER***

Lipids are ubiquitous biomolecules essential to all life, found in every cellular type, ranging from the human body and vegetal organisms, down to bacteria. They have many different functions in cell structuration, energy storage and signalling so they are natural biomarkers for different diseases like cancer, cardiovascular disease, neurodegenerative disease, lung disease, their study and quantification are then crucial. Mass spectrometry (MS) coupled with liquid chromatography or gas chromatography is mainly used for global and specific analysis of lipids. But before their analysis, there is an important and time consuming step of sample preparation. Due to their amphiphilic properties, there are usually two types of extractions: liquid-liquid extraction (LLE) and solid phase extraction (SPE). LLE and SPE are two very long protocols which are tedious when there are massive numbers of samples. They can also be the source of many errors, considering the experimenter-dependant possible repeated artefacts. To circumvent this point and increase the analytical service delivery, a TECAN robot has been acquired by MetaToul-Lipidomic facility to automate the sample preparation. Due to the specificity of lipid extraction, the robot needs a lot of optimization. This presentation will show part of adaptation we had to perform to validate fatty acid, neutral lipid and phospholipid profiling. That was related to the precision of solvent sampling which implies new adjustment accuracy for each of them, adding air gap, but also to modify the speed... First lipidomic's results obtained with a complete automated sample preparation will be presented for liver and plasma sample.

P-578 Novel analytical method for global metabolomic analysis by hydrophilic interaction/anion-exchange liquid chromatography tandem mass spectrometry

PRESENTING AUTHOR: ***Takeshi Bamba, Kyushu University, Japan***

CO-AUTHORS: ***Kohta Nakatani, Yoshihiro Izumi, Masatomo Takahashi, Keita Sakurai, Michio Butsugan, Takeshi Bamba***

Essential metabolic pathways to maintain biological activities include glycolysis, tricarboxylic acid cycle, amino acid, and nucleic acid metabolism. All intermediate of these metabolic pathways are polar ionic compounds. A profiling for these hydrophilic metabolites is the key to understand metabolic regulation. However, it is difficult to comprehensively measure them with a single analytical run by conventional analytical method such as reversed phase liquid chromatography (RPLC), hydrophilic interaction liquid chromatography (HILIC), or ion chromatography (IC), coupled with mass spectrometry. In this study, we developed a novel analytical method for global metabolic analysis using hydrophilic interaction/anion exchange liquid chromatography tandem mass spectrometry (HILIC/AEX/MS/MS). To construct a practical analytical system for global metabolic analysis, we developed a novel amino-polymer packed column, which was composed of polymer particles with amines as functional groups. The effect of the LC conditions on retention behaver and peak shapes of 45 representative hydrophilic metabolites was evaluated. As a result, the optimized mobile phase was 40 mM ammonium bicarbonate at pH 9.8 (A) and acetonitrile (B). After cationic and zwitterionic metabolites were eluted under HILIC condition (t = 1-15 min, 95-40%B), almost of all anionic metabolites were eluted under AEX condition (t = 15-16 min, 40-0%B; t = 16-26 min, 0%B). Thus, we named this chromatographic technique "HILIC/AEX". With the optimal method conditions established, we successfully identified approximately 200 hydrophilic metabolites from HeLa cell extracts. Taking all of the present results into account, our advanced analytical system will be useful tool for in-depth studies on global metabolism.

P-579 METABOLOMICS / LIPIDOMICS OF DRIED BLOOD SPOTS USING A NOVEL BLOOD MICROSAMPLING DEVICE

PRESENTING AUTHOR: ***Konstantinos Kouremenos, Trajan Scientific and Medical, Australia***

CO-AUTHORS: ***Konstantinos Kouremenos, Christopher Bowen, David de Souza, Kannan Ragunathan, Jason Hon, Dedreia Tull, Florian Lapierre, Anne Collins, Andrew Gooley***

Dried blood spots (DBS) offer many advantages over plasma, serum, and whole blood sampling, including being minimally invasive and easy to collect and store. DBS have been extended to applications such as therapeutic drug monitoring, pharmacokinetics, genomics, proteomics, lipidomics and metabolomics. A novel blood collection and storage device collects an accurate blood volume, controlled through the use of precision glass capillaries of a pre-determined volume (2.74 uL per capillary). The device provides 4 x 2.74uL replicates from the same blood source, which are then transferred to a blood storage substrate all encapsulated in a controlled desiccated environment to maintain sample integrity. Hence, the utility of the device is ideal for longitudinal metabolomic/lipidomic profiling. DBS samples prepared from the device were transferred into a 1.5mL Eppendorf tube (polar metabolomics), and another 1.5mL Eppendorf tube (lipidomics). One hundred microliters of chilled methanol was used to extract polar metabolites, with 100uL 1:1 butanol:methanol used to extract lipidic components. Targeted polar metabolite analysis was performed using a Shimadzu GCMS-TQ8040 (QQQ), while untargeted lipidomics analysis was completed using a Shimadzu LCMS 9030 (Q-TOF). Hundreds of metabolites were detected from one dried blood spot, including: glycolytic and TCA cycle intermediates, nucleosides, sugars, fatty acids, amino acids, organic acids. Similarly, hundreds of lipids were detected from one DBS, including classes: acyl carnitine, acylglucuronosyldiacylglycerol, bismonoacylglycerophosphate, cholesteryl ester, diacylglycerol, digalactosyldiacylglycerol, diacylglyceryltrimethylhomoserine, glucuronosyldiacylglycerol, hemibismonoacylglycerophosphate, ceramide, lysophophatidylcholine, lysophosphatidylethanolamine, monogalactosyldiacylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylserine, sphingomyelin, sphinganine, sphingosine, sulfoquinovosyl diacylglycerol, triacylglycerol.

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POSTER SESSIONS 3 AND 4 – Wednesday and Thursday – all even number posters will be on display.

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TECHNOLOGY

P-580 Exploring Plant Alkaloid Biosynthetic Pathways using Untargeted Metabolomics in Yeast

PRESENTING AUTHOR: ***Andreas Klitgaard, Novo Nordisk Foundation Center for Biosustainability, DTU, Denmark***

CO-AUTHORS: ***Lars Schrübbers, Mette Kristensen, Jie Zhang, Michael Krogh Jensen and Hanne Bjerre Christensen***

Plants are known producers of most potent human therapeutics as well as other industrially relevant molecules. Despite this wealth of compounds, very few products are commercially available, because of low production levels in the plants, as well as high price of production. The alkaloids are secondary plant metabolites that exhibit a remarkable structural diversity with several thousand alkaloids derived from a common precursor. Many of these also exhibit pharmaceutically valuable biological activities, which makes the large-scale production using recombinant microbial cell factories very attractive. Reconstitution of alkaloid biosynthetic pathways in a heterologous host is a proven strategy for rapid and inexpensive production of complex molecules. Baker's yeast Saccharomyces cerevisiae is one of the most common choices for a heterologous microbial host for plant metabolite production because of the abundance of available genetic tools for these organisms. However, a deep understanding of cellular metabolism is essential for their development. In this study, a Thermo Scientific ID-X mass spectrometer was used to investigate the cellular metabolism and to provide valuable insights into the changes associated with genetically modified biochemical reactions and metabolic pathways. Compound annotation was aided by use of the AcquireX workflow, enabling automatic selection and generation of target lists for LC-MS/MS analysis. Data Analysis was performed using Compound Discoverer 3.0, allowing for easy integration of LC-MS and LC-MS/MS data, and rapid compound annotation using a variety of different compound databases.

P-581 Semi-targeted UHPLC-MS metabolomic approaches: A way to make our life easier!?

PRESENTING AUTHOR: ***Lukáš Najdekr, Phenome Centre Birmingham, University of Birmingham, United Kingdom***

CO-AUTHORS: ***Lukáš Najdekr, Andrew Southam, Andris Jankevics, Martin R. Jones, Thomas Lawson, Giovanny Rodriguez Blanco, William Nash, Elliott Palmer, Mark R. Viant, Ralf J. M. Weber, Warwick B. Dunn***

A key challenge in mass spectrometry (MS) untargeted metabolomics is the annotation or identification of metabolite peaks. Metabolite identification must be performed, often manually, for each study individually which is time-consuming. Thus, there is a need for new and novel approaches to allow rapid identification of metabolites using MS, MS/MS and chromatographic retention time (RT) data. One way to address this bottleneck is through semi-targeted metabolomics. This involves the generation of a database of authentic chemical standards – containing RT and MS/MS data – collected using standardised ultra-high performance liquid chromatography-high resolution mass spectrometry (UHPLC-MS) methods. Here, we present three UHPLC-MS assays designed for the analysis of analytical batches of thousands of biological samples (serum, urine, tissue extracts). For the analysis of serum and tissue extracts, two complementary analytical assays (HILIC, C18 reversed phase) enable large coverage of the metabolome and lipidome. For urine, complementary HILIC and C18(aq) reversed phase assays are used. The data are acquired applying untargeted assays and a large proportion of the detected metabolites are automatically fully annotated/identified, which allows much more direct biological interpretation. Additional unidentified peaks are also collected and retained for statistical data analysis. Across the three assays, we have generated a database approaching a 1000 chemical standards containing the RT, full-scan MS and MS/MS data. Matching of the chemical standards to the biological data is performed using an automated data processing workflow. All analytical methods and data processing workflows will be made open-source to maximise the value to the wider scientific community.

P-582 Towards kit-based urinary metabolomics by GCxGC-TOFMS

PRESENTING AUTHOR: ***James Harynuk, Univeristy of Alberta, Canada***

CO-AUTHORS: ***Michael D.S. Armstrong, O. Rene Arreondo-Campos, A. Paulina de la Mata***

Comprehensive two-dimensional gas chromatography (GCxGC-TOFMS) is a valuable tool for metabolomics research. It offers vastly improved separation of components over one-dimensional methods, yielding purer spectra for compound identification and purer peaks for quantification. The major challenge in using GCxGC-TOFMS for quantitative metabolomic work lies in how to reliably perform and maintain instrument calibration for a large number of target compounds in a cost-effective and timely manner. In this pilot study, we use a group of 60 metabolites to evaluate a protocol for instrument calibration and for standard preparation that is easily adaptable to preparing suites of standards, potentially containing hundreds of analytes. Ultimately this will lead to sample preparation and calibration kits for performing urinary metabolomics studies by GCxGC-TOFMS, similar to those available for one-dimensional GC-MS.

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*AWARD WINNERS

TECHNOLOGY

P-583 Analysis of Lipid and Fatty Acid Isomers Using a Travelling Wave Cyclic Ion Mobility Separator

PRESENTING AUTHOR: ***David Heywood, Waters Corporation, United Kingdom***

CO-AUTHORS: ***Michael McCullagh, Martin Palmer, Russell J Mortishire-Smith, Johannes PC Vissers, Chris Hughes, James I Langridge***

Data were collected on a research platform based upon a SYNAPT G2-Si instrument where the standard T-Wave IM device is replaced by a cyclic ion mobility (cIM) separator. The cIM has multiple benefits: the circular path minimises instrument footprint whilst providing a longer, higher mobility resolution separation path; a multi-pass capability provides significantly higher IM resolution over a reduced (selected) mobility range; the device can be enabled for mobility separation or by-passed and the ion entry/exit array can selectively eject species. The cIM device consists of a 100 cm path length RF ion guide which provides mobility separation. MS and tandem MS data were obtained on precursor and product ion separated lipids, respectively, followed by TOF mass measurement. Unsaturated free fatty acid (FA) standards, differing in chain length and number of cis/trans conformations, were chosen to determine the degree of IM separation required to separate lipid isomers. FAs represent the simplest class of lipid components and are a core structural component of each lipid category. In direct infusion cIM-MS measurements FAs with cis-double bond orientations, introduced as two component mixtures, were found to be more compact than those with trans-orientations. Moreover, the cis- and trans-orientations for the monounsaturated FA's were distinguishable. A different number of cycles through the cIM separator were required to achieve a similar degree of IM separation for mono unsaturated FAs of differing chain length. Unsaturated FAs with two or more double bonds, separated by two mid-chain carbons, could not be distinguished.

P-584 Automated sample preconcentration into a (sub)microliter droplet using vision-controlled evaporation

PRESENTING AUTHOR: ***Paul Miggiels, Leiden University, Netherlands***

CO-AUTHORS: ***Paul Vulto, Isabelle Kohler, Thomas Hankemeier***

Analysis of volume- or biomass-limited samples, e.g. micro-dialysates, cerebrospinal fluid or cell culture extracts, has become essential for the discovery of new metabolite biomarkers. This, however, faces two significant challenges: i) low limits of detection required for low-abundant metabolites, and ii) dilution of small samples (<2 µL) during sample collection, handling and preparation. This study presents a novel preconcentration approach based on vision-controlled solvent evaporation from a constant-volume pendant droplet. This system is integrated in a commercial robotic autosampler for direct coupling to state-of-the-art LC-MS instrumentation. As the sample is dispensed from the autosampler's syringe, a pendant droplet forms at the needle tip inside a special evaporation chamber. The droplet volume is continuously monitored and maintained constant by adjusting the evaporation rate accordingly with a PID feedback loop. The chamber dimensions, temperatures and gas flow for evaporation have been optimized, achieving the preconcentration of 20 µL samples into 0.5-1.0 µL droplets in 3-5 minutes for various mixtures of water with common organic solvents. Repeatability has been evaluated for three model metabolites, i.e. leucine, tryptophan, and valine (N=4). Furthermore, 20-fold preconcentration of cell extracts, cell medium, and plasma samples was successfully demonstrated. By keeping the analytes in suspension during the preconcentration process, and making use of the self-cooling effect of evaporation, this technique is especially suited for volatile and thermosensitive metabolites. This novel approach opens new perspectives for high-throughput and exhaustive metabolomics analysis.

P-585 Ozone-Based Methods for Improved IM-MS Separations and Identification of Metabolites and Lipids

PRESENTING AUTHOR: ***Christopher Chouinard, Florida Institute of Technology, United States***

CO-AUTHORS: ***Robert Fraser-Caris, Kristie Baker, Samuel Maddox***

Ion mobility-mass spectrometry (IM-MS) has seen increased use in the fields of metabolomics and lipidomics during the last decade. In addition to the upsurge in commercially available IM-MS instrumentation, this increased use is primarily a result of the numerous advantages afforded by the technique: structure-based separations allowing for differentiation of isobars/isomers, measurement of collision cross section (CCS), etc. Despite these advantages, IMS separations remain challenged by limited resolution and inability to definitively identify unknown compounds in complex mixtures. This presentation will focus on ozone-based methods as a simple, cost-effective strategy for improving difficult IM-MS separations. Ozone-induced dissociation (OzID) is an established method for cleavage of C=C double bonds, allowing for precise determination of such bonds within fatty acid tails. Ozone-induced rearrangement (OzIR) is a recently introduced technique that targets C=C double bond-containing rings, such as those founds in steroids, prostaglandins, etc. Rather than producing m/z-specific fragments, OzIR changes the overall structure of a molecule, allowing for differentiation by IMS and MS/MS. This strategy has been successfully applied to several molecular classes, including isomeric prostaglandins A1 and B1. These structurally similar compounds differ only in the position of their double bond, and as such their MS/MS patterns are very similar and their CCS are nearly identical (187.0 and 185.4 Å2, respectively). However, following OzIR these isomers produce different fragmentation spectra and have a more than threefold increase in their ΔCCS, allowing effective IM-MS/MS differentiation. Herein we present improvements in metabolomics/lipidomics analyses using ozone-based methods.

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*AWARD WINNERS

TECHNOLOGY

P-586 Development of more reproducible and sensitive polar metabolomics methods

PRESENTING AUTHOR: ***Steven Fischer, Agilent Technologies, United States***

CO-AUTHORS: ***Sara Violante, Hardik Shah, Yuqin Dai, Justin R. Cross***

Ion-pairing reverse phase chromatography (IP-RPC) is a good separation technique for polar metabolites, but its usefulness is often constrained by signal suppression. Here, we reduce the signal suppression seen in IP-RPC using post-column additions that increase ionization efficiency, resulting in improved detection of metabolites over a wide dynamic range and from a single run. Cell extracts (HEK293 cells) and metabolite standards were analyzed using an IP-RPC method, with or without post-column chemistries, with a focus on chromatographic performance and signal suppression. The post-column chemistries consisted of acetone and dimethyl sulfoxide (negative mode) or diethylene glycol monomethyl ether (positive mode), added to the column effluent separately or together. Mobile phases and chromatographic gradients were optimized for each method considering several classes of biologically important compounds. Adding post-column chemistries increased the sensitivity of IP-RCP from >2-5 fold response. The highest increases in response were seen in nucleotides and other phosphorylated compounds in negative mode, as well as polyamines and B vitamins in positive mode. This required a 9:1 blend of acetone and DMSO (negative mode) or acetone and diethylene glycol monomethyl ether (positive more) and neither solvent achieved this effect when used alone. Other solvent blends were also tested with some success, but the effect on sensitivity was restricted to specific classes of compounds. We believe this increased sensitivity means IP-RPC should be considered more routinely for the separation and analysis of polar metabolites.

P-587 Improved High-throughput Targeted Lipidomic Analysis with sMRM Pro Builder

PRESENTING AUTHOR: ***Santosh Kapil Kumar Gorti, SCIEX, United States***

CO-AUTHORS: ***Sean Seymour, Mackenzie J Pearson, Christie Hunter, Paul RS Baker***

Isomer interference among different lipid classes is known as one of major challenges for application of LC-MS/MS methods for lipidomic analysis. Often, one lipid molecule can have multiple isomers and lipid classes sharing the identical precursor and product masses. To overcome this issue, an Amide chromatographic method is implemented to provide LC separation of lipid classes. In order to confirm separation efficiency, lipids standards (one standard per lipid class) were injected individually to confirm that there was no isomer crosstalk among different lipid classes. Preliminary data is collected with no MRM scheduling on a representative matrix (with or without internal standards) for any biological study. Results from this unscheduled MRM datasets are entered into the sMRM Pro Builder and an initial rough approximation of the retention times is determined. Next replicate injections are performed on the matrix using a preliminary retention time scheduled MRM method, ideally with a pooled sample from all the biological samples. Data analysis here provides information on peak width, RT variance, lipid signal, then the sMRM Pro Builder computes a final optimized time scheduled MRM method. Excellent reproducibility was observed with majority of lipid species showing retention time standard deviations below 0.05 minutes. Lipid standards from 19 different classes, which are either heavy isotopic labeled lipids or odd chain lipids, were used as internal standard. This method provided extensive lipid class coverages including, CE, CER, DCER, HCER, LCER, TAG, DAG, MAG, LPC, PC, LPE, PE, LPG, PG, LPI, PI, LPS and PS.

P-588 Improving lipid annotation coverage using intelligent precursor selection software on an Orbitrap-based mass spectrometer

PRESENTING AUTHOR: ***Elizabeth Crawford, Thermo Fisher Scientific GmbH, Germany***

CO-AUTHORS: ***Sven Hackbusch, David Peake, Reiko Kiyonami***

Lipid profiling provides valuable information to identify disease states and other physiological changes. A common approach used in lipid profiling by LC-MS is to identify lipid species by their MS/MS spectra prior to extraction of precursor information for relative quantitation between conditions. The novel intelligent data acquisition strategy, AcquireX, on the Thermo Scientific™ Orbitrap ID-X™ Tribrid™ mass spectrometer automatically excludes background ions from the MS/MS acquisition and prioritizes MS/MS precursor selection on sample relevant compounds, resulting in deeper lipidome coverage. Here, Bovine liver total lipid extract (Avanti Polar Lipids) was used to demonstrate the utility of the workflow. Samples were separated using reversed-phase conditions on a C30 column with mass spectral data acquired on an Orbitrap ID-X Tribrid MS. Data analysis was performed using Thermo Scientific™ LipidSearch™ 4.2 software to identify lipid molecular species based on acquired MSn fragmentation spectra. The AcquireX data acquisition strategy was used to automatically generate background exclusion and/or compound inclusion lists that were updated iteratively for replicate injections, reducing redundant data collection and triggering more unique lipids for fragmentation. As a result, a significantly larger number of lipids (>40%) could be detected compared to a conventional data-dependent MS/MS approach. In addition, the acquisition of neutral loss-triggered CID-MS3 and product ion-triggered CID-MS2 improved the confidence in the lipid annotations. For example, the use of fatty acid neutral loss-triggered MS3 fragmentation with triglycerides allowed to distinguish multiple co-eluting isomers with different fatty acid chain lengths based on their respective MS3 data.

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*AWARD WINNERS

TECHNOLOGY

P-589 Intelligent Acquisition for Comprehensive Metabolome Coverage in Plants, Mammals, and Bacteria

PRESENTING AUTHOR: *Tatjana Talamantes, Thermo Fisher Scientific, United States*

CO-AUTHORS: *Sven Hackbusch, Ioanna Ntai, Amanda Souza*

Compound identification remains a challenge in untargeted metabolomics. In LC/MS based untargeted metabolomics experiments, the detection of thousands of features in a single sample is routinely accomplished. However, this should not be equated to “global” metabolome coverage, as only a small percentage of those metabolites are of biological origin. Data-dependent acquisition (DDA) often provides information for the most abundant ions. Recently developed AcquireX acquisition software can determine on-the-fly features corresponding to background contaminants and compound degeneracy, such as isotopes, adducts, and dimers, enabling more efficient MS/MS and MSn sampling of unique biologically relevant metabolites. Unlike traditional DDA, during which the fragmentation of background ions dominates the duty cycle, the AcquireX workflow selects precursors intelligently by excluding background ions and targeting unique metabolites of biological relevance for fragmentation. Here, we used samples of varying matrix and complexity, to demonstrate the utility of AcquireX acquisition across several sample types. By excluding background and degenerate signals, the total number of fragmentation targets was reduced without compromising metabolite coverage. By focusing acquisition on biologically relevant compounds, more time could be spent collecting multistage (MSn) fragmentation data, without affecting experiment length. MSn provided additional structural information and confidence for compound annotations and, in the case of flavonoids, isomeric compound annotation candidates could be differentiated without the need for additional experiments. Ultimately, AcquireX intelligent acquisition enabled annotation of non-biological and redundant features on-the-fly, resulting in comprehensive MSn coverage regardless of sample type, complexity, and concentration. For Research Use Only. Not for use in diagnostic procedures.

P-590 Development of a Collision Cross Section Library using Trapped Ion Mobility Spectrometry (TIMS) and Its Use in Plant Metabolomics

PRESENTING AUTHOR: *Mark Schroeder, University of Missouri - Columbia, United States*

CO-AUTHORS: *Sven Meyer, Aiko Barsch, Lloyd W. Sumner*

The timsTOF has been frequently used in the analysis of proteomics and is now gaining increased interest for small-molecule metabolomics. To evaluate the potential benefits the TIMS technology can provide, we have measured the CCS values of plant specialized metabolites such as flavonoids and saponins. These compounds are structurally diverse due to various flavonoid and triterpene aglycones as well as various modifications to the aglycones such as hydroxylation and glycosylation. We recorded CCS values for over 150 specialized metabolites. Specifically, the samples were analyzed in triplicate and the average, standard deviation, and relative standard deviation (RSD) of CCS values were recorded. RSD of <1.0% were obtained for all compounds. In addition, we annotated adduct formations of the metabolites which are commonly observed in LC-MS analysis. To test the robustness of the CCS measurements and matching of library compounds to analytes we further tested a standard mix of 5 compounds from our library. We pooled 5 flavonoids and glycosylated flavonoids and analyzed with and without a plant compound matrix through direct infusion TIMS-QTOFMS and UHPLC-TIMS-QTOFMS. Then we compared the recorded CCS values between the various methods and the recorded library. We found the CCS measurements and the library were consistent and the compounds could be reproducibly detected and identified during data processing. The usefulness of the CCS library was demonstrated in the analysis of Medicago truncatula and Glycine max extracts. Results indicated that the CCS library allowed for more confident identifications of flavonoids and saponins with improved annotation quality scores.

P-591 Simultaneous analysis of SIM and Scan mode in a single run using LC-QTOFMS: Comprehensive cell culture profiling of iPS cell

PRESENTING AUTHOR: *Takanari Hattori, Shimadzu Corporation, Japan*

CO-AUTHORS: *Toshiya Matsubara, Tsuyoshi Nakanishi, Jun Watanabe*

Culture medium is composed of various biologically important compounds such as vitamins, amino acids, nucleic acids and other primary metabolites. Comprehensive analysis of these compounds would lead to more detailed understanding of the bioprocess. We report comprehensive cell culture profiling of iPS cell by metabolomics based LC-QTOFMS. SIM and Scan mode were simultaneously used in a single run. Feeder-free iPS cells (1231A3) were maintained in AK02N medium for 6 days. Proteins were removed from the supernatants by adding acetonitrile and centrifugation. The supernatants were analyzed after dilution with ultrapure water. NexeraTM X2 system coupled with a LCMSTM-9030 (Shimadzu Corporation, Japan) was used. MS analysis consisted of SIM and Scan mode in positive mode. 68 compounds were targeted for SIM mode. MS spectra were acquired from m/z 50 to 500 for Scan mode. As a result of targeted analysis using SIM mode, 27 compounds such as amino acids and vitamins were detected with high sensitivity. Alanine, kynurenine, ornithine and few compounds have increased with time course. In contrast, arginine, methionine, tryptophan and few compounds have decreased with time course. As a result of untargeted analysis using Scan mode, some unknown compounds were detected and their amount have increased or decreased with time course. Accurate mass, database (Human Metabolome Database) and MS/MS spectrum were used to identify unknown compounds and N’-formylkynurenine was identified as one of unknown compounds. N’-formylkynurenine is intermediate metabolite between tryptophan and kynurenine in kynurenine pathway. N’-formylkynurenine have increased with time course.

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*AWARD WINNERS

TECHNOLOGY

P-592 HILIC-HR-MS for (untargeted) metabolomics in microorganisms – the optimal method for polar compounds in an industrial setting?

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CO-AUTHORS: *Wouter Coppes, Reza Maleki-Seifar, Sandra Pous-Torres, Raymond Ramaker*

The analysis of polar intracellular metabolites, e.g. amino acids, organic acids, sugar phosphates and nucleotides, is always a challenge. Although for each class of compounds separate methods are available, there are not many methods that can cover all these classes at once. One of the methods that can do this is ion-pair liquid chromatography. Ion-pair LC gives very good retention for polar metabolites on C18 columns. The major drawback of the use of an ion-pair agent is the contamination of the MS, especially when MS instruments are shared and used for different purposes. This often means that the only option to use ion-pair LC is having a dedicated LC-MS system that is only used for this purpose. However, this might not always be possible and when possible, there is not always high-end LC-MS equipment available. There was therefore a big need in our lab to find a good alternative for ion pair LC. The aim was to have a method that is compatible with high-end HR-MS equipment. Hydrophilic interaction liquid chromatography (HILIC) is often proposed as good alternative and many papers have described the possibilities of HILIC for the analysis of polar intracellular metabolites. In this poster we will describe our search for the best HILIC-HR-MS method for intracellular polar metabolites that could replace our ion-pair LC-MS method. Different columns, buffers, pH etc. were studied and, very important, the method was tested using real-life complex matrices and samples.

P-593 A high throughput liquid chromatography-mass spectrometry metabolomics method and its application in early warning of diabetes

PRESENTING AUTHOR: *Yang Ouyang, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, China*

CO-AUTHORS: *Xinjie Zhao, Guowang Xu*

Metabolomics has been widely applied in clinical study recently. For conventional metabolomics methods based on liquid chromatography-mass spectrometry (LC-MS), procedures of pretreatment and separation are time-consuming. And it directly influences the sample throughput. To fulfill the requirement of the large-scale sample analysis, a high throughput metabolomics method was established by using an efficient 96-well-plate-based sample preparation protocol and a short 12-min LC gradient, which reduced about 70% of the sample processing time and 60% of the analysis time. Evaluation of analytical performances including the coverage of metabolites reflected that the method was robust for the large-scale metabolomics study. To show the usefulness, in a nested case-control study comprising 30,000 Chinese participants, subjects with new-onset diabetes in five years were selected as a case group (n=295), matching the subjects of the healthy group with no diabetes in the same period according to clinical information such as age and gender (n=295). Using this high throughput method, we found that amino acids, acylcarnitine and unsaturated free fatty acids accumulated in the case group, while the amount of lyso-phosphatidylcholine species decreased. And differential metabolites' combination also achieved a suitable discriminating power between the control and case. Therefore, our research has the potential to provide a research basis for the early warning of diabetes. Moreover, the high throughput method has a wide application prospect in the field of large-scale metabolomics study.

P-594 Data Stability of GC-FID in Metabolomics

PRESENTING AUTHOR: *Takero Sakai, Shimadzu Corporation, Japan*

CO-AUTHORS: *Yusuke Takemori, Kiyomi Arakawa*

One of the most important and difficult problem in Metabolomics is data stability. In GC-MS Metabolomics, the data would be unstable as time passed after TMS derivatization, that might be caused by MS tuning, pollution, and the stability of derivatized compounds. Therefore in GC-MS, data acquisition with long time span may sometimes need some modification to the data, such as correction with quality control sample. GC-FID is a conventional analytical instrument that is useful for many fields. However GC-FID might be less utilized in Metabolomics than GC-MS so far. This is because GC-MS is more powerful in peak annotation and peak annotation is considered necessary for Metabolomics. But GC-FID has a massive feature for Metabolomics; data stability. We prepared commercial product of beer as samples and try to analyze it with GC-FID. The stability of the obtained data within 60 hours were incredibly better than GC-MS. Moreover, we stored the extraction of the samples with dryness and analyzed it after 2 weeks. The obtained data after 2 weeks were almost identical not only as chromatogram shape but also peak area. Under these background data, we tried to classify the subtle beer samples that were the same as product but differentiate with their lots and manufacturing place. With these result, we suggest that GC-FID has an underrated potential for Metabolomics, especially for classification / regression of samples with big data that needs more stable data acquisition system than MSs.

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TECHNOLOGY

P-595 Chemical isotopic labeling of multiple functional groups in urine metabolome

PRESENTING AUTHOR: ***Cheng-Yu Hung, Chang Gung Molecular Medicine Research Center, Taiwan***

CO-AUTHORS: ***Cheng-Yu Hung, Ya-Ju, Hsieh, Jau-Song Yu, Yi-Ting Chen***

A high proportion of metabolites in human urine possess hydroxyl and amino groups. Chemical Isotope Labeling (CIL) is useful for improving the poor ionization efficiency and low existing level in biological fluids by liquid chromatography-mass spectrometry (LC-MS). Dansyl chloride (DnsCl) has been widely reported as a derivatizing reagent for facilitating the MS detection. We described a developed dansylation method for improving the mass spectrometry detection of the amine/phenol and the hydroxyl metabolites in the urine samples. To optimize the whole workflow, we measured with five reaction factors including sample diluted factors, reaction volume, reaction time, reaction temperature, and molar ratio of DnsCl to catalyst. A urinary pooling was labeled with 12C- or 13C-DnsCl on a base-activated reaction. Chemical standards of amino acid and hydroxyl compounds were constructed for positive control. After incubation at 65 for one hour, the equal mole amount of 12C- / 13C-labeled mixtures in triplicate were analyzed the peak pairs of labeled metabolites using LC-MS. Furthermore, we also compared this developed method with other dansylation protocols. In summary, this technique improves the detection for labeling both amino group and hydroxyl group simultaneously.

P-596 MetaboLights study editor - An open-access curation tool for metabolomics studies submission and associated meta-data annotation

PRESENTING AUTHOR: ***Venkata Chandrasekhar Nainala, The European Bioinformatics Institute (EMBL-EBI), United Kingdom***

CO-AUTHORS: ***Claire O'Donovan, Jiakang Chang, Jose Ramon Macias, Kalai Vanii Jayaseelan, Keeva Cochrane, Kenneth Haug, Namrata Kale, Pablo Moreno, Rachel Spicer, Mark Williams***

MetaboLights database is an international metabolomics repository recommended by many leading journals including Nature, PLOS and Metabolomics. The service's unique manual curation maintains quality, provides helpful support for users and ensures accessibility for secondary analysis of studies. MetaboLights hosts a wealth of cross-species, cross-technique, open access experimental research. As a part of our ongoing efforts to streamline the study submission and curation process, the MetaboLights team at EMBL-EBI has developed a new tool to submit and edit studies online. This submission tool provides MetaboLights users and curators with an intuitive and easy to use interface to create, edit and annotate their studies online. The convenient, context-aware editor navigates users through the study to define a rich description of the experimental metadata including study characteristics, protocols, technology and related factors. Metadata descriptions are enhanced by mapping this information to controlled ontologies repositories using ZOOMA, capturing such a complete data set benefits the community by making results findable, reproducible and reusable. Going forward we have plans to incorporate text mining tools such as Named Entity Recognition (NER) to annotate metadata, enabled by the robust architecture of the online editor. Other ideas include offline edit support, direct channels for curators to contact and communicate with the submitters to make the whole process of data curation more submitter-friendly.

P-597 Effects of Deuterium Oxide (D2O) in 1H NMR Metabolomics

PRESENTING AUTHOR: ***Kristina Haslauer, Helmholtz Zentrum Munich, German Research Center for Environmental Health, Germany***

CO-AUTHORS: ***Daniel Hemmler, Philippe Schmitt-Kopplin, Silke Heinzmann***

Sample preparation in urine NMR metabolomics generally requires only few steps, however, preparation protocols still have not reached consensus. Most variability in the protocols is given by varying D2O concentrations as locking substance in buffer systems and temperature handling during dwell time prior to measurement. We investigated the effect of deuterium oxide concentrations on urine metabolites for different temperature conditions and found a successive decrease in creatinine peak area up to 35% after 24 h. Creatinine is known to be excreted in a constant rate over 24 h in healthy individuals and therefore frequently used as normalization factor for urinary dilution. Furthermore, creatinine is used as important biomarker for renal function. To address this loss in peak area, a systematic investigation on the underlying mechanism and the impact was carried out in this study. A proton-deuterium (H/D) exchange at the CH2 position was revealed by 1H, (IG) 13C, DEPT-HSQC NMR and MS experiments leading to this loss. Furthermore, we conducted a sample stability examination for different D2O concentrations and temperatures up to 24 h. We propose an equation to correct the creatinine loss for biobank samples, which was validated on an external dataset, as well as a general guideline for future studies to ensure a high creatinine stability.

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TECHNOLOGY

P-598 Impact of pre-analytical sample handling on plasma for 1H-metabolomics

PRESENTING AUTHOR: ***Anders Pedersen, Swedish NMR Centre at the University of Gothenburg, Sweden***

CO-AUTHORS: ***Daniel Malmodin, Huma Zafar, Åsa Torinsson Naluai, B. Göran Karlsson***

The pre-analytical sample handling of plasma samples prior to freezing has an unwanted impact on resulting metabolite concentrations in bio-banked samples. It is important to quantify how various metabolites are affected depending on handling since it otherwise risk to bias studies when e.g. study samples and controls have not been treated equally. Using 1H NMR spectroscopy we have investigated the effect on the metabolite profile by exposing plasma samples to light or no light, three different temperatures and different times prior to centrifugation. While light or no light does not influence the metabolite concentrations, temperature and time to centrifugation does, in a predictable way. Therefore, it would have been preferable if temperature and time to centrifugation values had been stored when samples were collected. Since this usually is not the case, an alternative approach is to estimate these parameters from relative concentration differences between specific temperature and time sensitive metabolites. We present sensitive metabolites and outline how their concentrations can be used to estimate the exposure to temperature and time to centrifugation for a given sample.

P-599 Development of a Metabolomics System Suitability Sample for MS-based Metabolomics

PRESENTING AUTHOR: ***Tracey Schock, National Institute of Standards and Technology, United States***

CO-AUTHORS: ***Deb Ellisor, Clay Davis***

Prior to the actual analysis of biological samples, one must first assess the suitability of the analytical instrumentation. In this regard, the metabolomics community is suffering from the lack of an everyday system suitability standard by which to benchmark instrument performance for untargeted MS based approaches. A complex solution that mimics biological samples is required to determine whether the analytical run is of acceptable quality and to ensure lack of contamination prior to experimental analyses. Individual laboratories use a small number of standards (5-15) to create in-house suitability solutions for assessing measurement quality of hundreds to thousands of chemicals profiled in an untargeted study of incredibly complex samples. Over-reporting and spurious conclusions are likely rampant in the literature due to the lack of a material to evaluate measurement quality across a complex omics profile. NIST is developing a large quantity, biological extract from human liver which incorporates the entirety of a metabolome, resulting in a more encompassing system suitability sample. The design of a tissue extract as a suitability standard eliminates sample preparation variation observed with biological samples while offering simplicity of use and analyte complexity for analysis of metabolomics platforms. Additionally, the extract can be a tool in harmonization of instrument performance in large, multi-center studies. NIST candidate research grade material (RGM) 10122 Metabolomics System Suitability Sample will be assessed for homogeneity and stability of constituent metabolites. The sample is available pre-sale for those interested in evaluating the material for the metabolomics community's needs.

P-600 mQACC: A community-led initiative to promote quality assurance and quality control in untargeted metabolomics research

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CO-AUTHORS: ***Fadi Abdi, Abbas Bandukwala, Aiko Barsch, Dan Bearden, Richard Beger, Bianca Bethan, David Broadhurst, Clary Clish, Surendara Dasari, Leslie Derr, Suraj Dhungana, Warwick Dunn, Tim Ebbels, Annie Evans, Steve Fischer, Roberto Flores, Thomas Flynn, Charles Grieser, Thomas Hartung, Majda Haznadar, David Herrington, Rick Higashi, Ping-Ching Hsu, Christina Jones, Judith Jans, Maureen Kachman, Jennifer Kirwan, Andre Kleensang, Matthew Lewis, Katrice Lippa, Padma Maruvada, Sven Meyer, Maria Eugenia Monge, Jonathan Mosley, Ioanna Ntai, Claire O'Donovan, George Papanicolaou, Rui Pinto, Mary Playdon, Dan Raftery, Sharon Ross, Michael Schmidt, Tracey Schock, Amanda Souza, Jinchun Sun, Fariba Tayyari, Georgios Theodoridis, Frederico Torta, Baljit Ubhi, Vidya Velagapudi, Mukesh Verma, Mark Viant, Dajana Vuckovic, Li-Rong Yu, Tilmann Walk, Ian Wilson***

The metabolomics Quality Assurance and quality Control Consortium (mQACC) is a community-led initiative to promote quality assurance (QA) and quality control (QC) in untargeted metabolomics research. mQACC was established through a National Institutes of Health-funded meeting in October 2017. Data from a survey published by Dunn, et al. Metabolomics, 2017) was used to establish meeting objectives: 1.) identify the most useful metrics for assessing study and data quality for untargeted metabolomic studies; 2.) identify and prioritize processes to ensure appropriate reporting of QA/QC data; and 3.) identify and prioritize the types of test materials that are needed in the field of metabolomics for QA/QC in untargeted studies. Key priorities were identified and scored for importance, resulting in several primary themes. Although priorities were identified for long-term efforts, three immediate priorities were moved forward: 1.) publish a workshop report (Beger et al., Metabolomics, 2019); 2.) document and subsequently publish the complete experimental procedure for untargeted metabolomics; and 3.) identify 2-3 reference materials to be developed quickly. mQACC currently includes over 55 representatives from the North America, South America, Europe, and Asia, including instrument manufacturers, commercial laboratories, and government and academic stakeholders. The consortium is currently addressing their initial priorities, as well as expanding these efforts. mQACC has also established several working groups to achieve their objectives, including ones focusing on experimental procedures, reference materials, QC best practices, and reporting standards. In addition to describing the mQACC priorities, we will highlight how the metabolomics community can get involved in this important initiative.

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TECHNOLOGY

P-601 Metabolomics with Matrigel-based 3D cell culture: analytical challenges

PRESENTING AUTHOR: *Janina Tokarz, Helmholtz Zentrum München, Research Unit Molecular Endocrinology and Metabolism, Germany*

CO-AUTHORS: *Gabriele Möller, Cornelia Prehn, Jerzy Adamski*

Cell culture metabolomics is a versatile tool to understand metabolic processes in cells. Two-dimensional (2D) cell culture allows for easy manipulation of cells and is well established in metabolomics experiments. However, cells in 2D encounter a non-physiological environment and are known to loose key functionalities such as their xenobiotic metabolizing capacity. These limitations can be overcome by cultivating the cells in three-dimensions (3D) supported by extracellular matrices such as Matrigel. Our goal was to analyze the feasibility of combining Matrigel-based 3D cell culture with targeted metabolomics. Hep G2 cells were seeded in varying densities into Matrigel. Solidified Matrigel-cell complexes were covered with culture medium. The cell complexes were harvested by scraping them in ice-cold extraction solvent consisting of 80% methanol and subsequently disrupted using bead-based homogenization. After centrifugation, metabolites were analyzed with the Biocrates AbsoluteIDQTM p180 Kit. 2D cultured cells and plain Matrigel samples were analyzed in parallel. We observed significant ion suppression by Matrigel. In addition, some metabolites, mostly lipids, showed higher concentrations in cell than in Matrigel samples. Many amino acids and the hexoses were higher concentrated in Matrigel samples without cells, indicating diffusion of these metabolites from the culture medium into the matrix. Our data demonstrate that targeted metabolomics with Matrigel supported 3D cell culture samples is possible. However, due to the matrix, metabolites provided by culture media remain in the sample despite washing steps. Thus, the extracellular matrix causes a major contamination and remains a challenge for metabolomics experiments.

P-602 Feature-Based Molecular Networking of Non-targeted Mass Spectrometry Data: Bridging MS-DIAL, MZmine2, OpenMS, and XC-MS, with the GNPS web-platform

PRESENTING AUTHOR: *Louis Felix Nothias, University of California San Diego, United States*

CO-AUTHORS: *Louis Felix Nothias, Daniel Petras, Mingxun Wang, Robin Schmid, Abinesh Sarvepalli, Zheng Zhang, Ricardo da Silva, Alexander Aksenov, Pieter C. Dorrestein*

Molecular networking has become an essential bioinformatic tool to annotate non-targeted tandem mass spectrometry data (MS/MS). Available on the GNPS web-platform (http://gnps.ucsd.edu), molecular networking accelerates the annotation of molecular/spectral families by propagating spectral library matches across the networks. Based on the MS-Cluster algorithm, molecular networking enables the large scale metabolomics meta-analysis, up to thousands of files. However, as MS-Cluster uses the MS/MS spectral counts as a proxy for the ion distribution between samples, other methods are needed to estimate the relative ion abundance, especially when analysing a single study. In the present work, we introduce feature-based molecular networking (FBMN), a collection of computational tools integrated together into a seamless LC-MS data processing pipeline combined with molecular networking on the GNPS web-platform (https://ccms-ucsd.github.io/GNPSDocumentation/featurebasedmolecularnetworking/). Utilizing software with advanced graphical user interface (MS-DIAL, MZmine2, MetaboScape) or advanced computational libraries (OpenMS, and XC-MS), the workflows serves both experimentalists, bioinformaticians, and software developers. We benchmarked FBMN on dilutions of the NIST serum samples, and results showed that it improves the estimation of ion relative distribution between samples, and reduced considerably the number of spectral artefacts. These results were observed for both Orbitrap and QTOF instruments, and different sample types, indicating that FBMN has significant advantages over MS-Cluster based molecular networking. Nonetheless, the FBMN is proposed to serve as a complementary tool to the “classical”, MS-Cluster-based molecular networking which is a robust and nearly parameterless method, and is the only method capable of performing large scale meta-analysis of non-targeted LC-MS/MS metabolomics datasets.

P-603 A comparison of untargeted metabolomic methods applying 2.1 and 1.0 mm ID UHPLC columns for the study of mammalian metabolomes

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CO-AUTHORS: *Lukàš Najdekr, Mark R. Viant, Caroline A. Rowland, Warwick B.Dunn*

Untargeted ultra-high-performance liquid chromatography-mass spectrometry (UHPLC-MS) metabolomics has been successfully applied for the study of a wide variety of sample types. Standard methods employ columns with 2.1mm internal diameters (ID) where sample volume is available in sufficient quantities. However, if sample volumes are limited (<10µL), more sensitive analytical methods are required because of the requirements for sample dilution. Previous research has shown that use of a smaller ID column provides the required increase in sensitivity for a limited number of sample types and complete characterization of sensitivity improvements has not been performed (Gray et al., 2016, Gray et al., 2015, Plumb et al., 2004). Therefore, the aim of the study presented here was to determine the influence of column ID on sensitivity and volume of sample required by comparing 2.1mm ID column and 1.0mm ID column methods. Human plasma and urine were prepared in serial dilutions and UHPLC columns of two different IDs (2.1 and 1.0mm) but with the same stationary phase composition and particle size were used to optimize the analysis. Three different analytical assays were used: lipophilic reversed phase C18, aqueous end-capped reversed phase C18 for semi-polar metabolites and hydrophilic interaction chromatography (HILIC) for polar metabolites. The 1.0mm methods were then applied to the analysis of mammalian tear samples which are available in volumes of <10µL. Results show that following optimization, the 1.0mm ID methods are more sensitive than the 2.1mm ID methods for the same sample volume and are appropriate for untargeted mammalian tear analysis.

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TECHNOLOGY

P-604 A simple strategy to improve data quality in LC-MS-based metabolomics studies of human urine

PRESENTING AUTHOR: ***Adriana Nori de Macedo, Federal University of Minas Gerais, Brazil***

CO-AUTHORS: ***Rosilene Cristina Rossetto Burgos, Pedro Luis Rocha da Cruz, Helio Tedesco-Silva Junior, Karina Helena Morais Cardozo, Valdemir Melechco Carvalho, Marina Franco Maggi Tavares***

An important challenge in metabolomics when using liquid chromatography-mass spectrometry (LC-MS) is the variation of signal intensity during sample analysis, due to accumulation of sample components in the column and front parts of the MS system. Herein, we suggest simple changes to improve data quality in LC-MS-based metabolomics, which were tested for urine samples (n=176) from renal transplant patients. These changes include the use of a divert valve to direct the column effluent to waste at the beginning of the chromatographic separation (0.0-0.8 min) and during column cleanup and equilibration (12.0-20.0 min), combined with a longer column cleanup between injections (6.0 min at 0.6 mL/min, instead of 1.0 min at 0.4 mL/min). The method was tested before and after introducing these modifications by analyzing individual urine samples, quality controls (QCs), blanks, and calibrant solutions using an UPLC (Waters) and QExactive (Thermo), along with Skyline for data monitoring. QCs showed great improvement in peak area repeatability, with relative standard deviations (RSDs) of selected metabolites going from ~60% to ~10% as the divert valve and longer column cleanup were introduced. Similarly, RSDs of peak areas for internal standards spiked into individual urine samples improved from ~40% to ~10%. Moreover, calibrant solutions injected firstly and lastly in the analytical sequence had more comparable signals after introducing the modifications. Therefore, in addition to current protocols of preventative maintenance prior to sample analysis, we recommend the use of a divert valve and extended column cleanup as simple modifications to improve data quality in metabolomics studies.

P-605 High-throughput Targeted Lipidomics Analysis of Dihydroceramide Desaturase-1 (DES1) Knockout Mice

PRESENTING AUTHOR: ***Mackenzie Pearson, SCIEX, United States***

CO-AUTHORS: ***Santosh Kapil Kumar Gorti, Trevor S. Tippetts, Scott A. Summers***

Amide column chemistry was chosen for lipid class separation and minimize isomeric interference. The target list of lipids is comprehensive, covering most major lipid classes and categories, and MRMs were selected to cover lipids containing fatty acids with 14 to 22 carbons and 0 to 6 double bonds. The method is customizable, so new lipid categories, classes and molecular species can be added to the MRMs list. This method provides quantitative measurement of over 1150 different lipid molecular species in a rapid, highly reproducible manner. The sMRM Pro Builder template which was developed to streamline the method optimization process, enables assigning the retention time, optimize dwell weight and set window size per MRM to enhanced coverage and sensitivity of the method. This optimization improved results quality especially on low abundant lipids. Liver and eWAT tissues were harvested from dihydroceramide desaturase-1 (DES1) knockout mice. DES1 is the enzyme responsible for inserting the 4,5-trans-double bond into the sphingolipid backbone causing the dihydroceramide conversion to ceramide. While both of these classes of lipids are lower in abundance in the chosen tissues, this method shows significant changes in these lipid classes in a quantitative manner. However, lipids from another 17 classes were not changing. Lipid standards from 19 different classes, which are either heavy isotopic labeled lipids or odd chain lipids, served as internal standard. This method provided extensive lipid class coverages including, CE, CER, DCER, HCER, LCER, TAG, DAG, MAG, LPC, PC, LPE, PE, LPG, PG, LPI, PI, LPS and PS.

P-606 A novel approach of untargeted plant secondary metabolic profiling and its application to studying resistance-related metabolites in maize

PRESENTING AUTHOR: ***Zaifang Li, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, China***

CO-AUTHORS: ***Xiuqiong Zhang, Yueyi Xia, Xiaoshan Sun, Wenying Liang, Yaorui Ye, Chunxia Zhao, Xin Lu, Guowang Xu***

Secondary metabolites are the end products of complex metabolic pathways in plants. They are widely involved in physiological processes such as growth, development, and defense. Plants fight against biotic and abiotic stress by synthesizing and releasing defensive secondary metabolites. However, comprehensive identifications of secondary metabolites are difficult because of plant chemical diversity, highly species/tissue-specific and time-dependent accumulations. It is necessary to develop a comprehensive analytical method for secondary metabolites. In this study, a novel metabolic profiling method based on ultra-high-performance liquid chromatography–high-resolution mass spectrometry was established and applied to investigate secondary metabolites of maize. A high coverage MS/MS acquisition method was developed by a segmented scan. Then, a multi-informational molecular network was constructed for comprehensive identification of secondary metabolites in maize. The secondary metabolites composition of maize leaves, silk and seeds were investigated using the developed method. A tissue-specific accumulation of secondary metabolites was observed. Herbivore-induced alterations in secondary metabolites of maize silk were studied. We found that a great variation was observed on the secondary metabolism of maize silk after insect attack. The insect-resistant metabolites, benzoxazinones, phytoalexins belonging to terpenes (KB1, KA2, KA3, ZB1 and dolabradiene, etc.), hydroxycinnamic acid amides and its precursor cinnamic acids were significantly accumulated in maize silk. While flavonoids and its flavonoid phytoalexins (maysin) were significantly decreased after the attack. These results show the developed untargeted metabolic profiling method is very useful for novel insights into secondary metabolites defense in crops.

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TECHNOLOGY

P-607 Deeper Level of Comprehensive Metabolite Quantitation with Data Independent Acquisition

PRESENTING AUTHOR: ***Baljit Ubhi, SCIEX, United States***

CO-AUTHORS: ***Zuzana Demianova***

The major challenge in the field of metabolomics is to accurately identify and quantify hundreds of metabolites in a single run. Recently variable window SWATH acquisition has shown to identify a higher number of metabolites compared to the traditional Data Dependent Acquisition (DDA) approach, thus enabling broader metabolome coverage. Here we have implemented a variable window SWATH acquisition method for enhanced quantitation of selected metabolites using MS/MS, with reduced matrix interferences and improved signal-to-noise. Using MS/MS fragments for metabolite quantitation provides better selectivity, and ultimately increased sensitivity. Variable window SWATH Acquisition provided quality quantitative data for metabolites in complex matrix. Due to many coeluting metabolites in complex matrix, using only the MS spectrum and retention time is often not sufficient for metabolite identification. MS/MS information is necessary to obtain further structural knowledge about the metabolite. Complete full scan MS and MS/MS data is available in every SWATH file for improved ID. In addition, MS/MS quantitation of metabolites often leads to lower detection limits due to significantly improved signal to noise ratios vs MS data. Measuring the whole MS/MS spectrum allows selection of the best fragments for metabolite quantitation. SCIEX OS software combines comprehensive qualitative and quantitative data analysis, making data processing easier and more efficient. SWATH Acquisition on all detectable metabolites is successfully utilized for identification, and accurate MS/MS level quantification of metabolites in urine.

P-608 Automated High-Throughput Flux Analysis of Non-Small Cell Lung Carcinoma Cells Grown in vitro in Two and Three Dimensions

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Cell lines are widely used to study disease and treatment. Monolayer (2D) cell cultures are commonly used for this purpose. The geometry of these cell cultures is however believed to be inadequate to recreate the growth environment of cells. Spheroid (3D) cell cultures are considered to be more viable in vitro alternatives since they better mimic the in vivo conditions. The different outcomes from 2D and 3D culture studies can therefore have a significant impact on the relevance of experimental findings. H1299 cells were grown as 2D monolayers in Eagle's media, and to generate 3D spheroids, cells were grown in 2% pHEMA dissolved in ethanol. Following sample preparation, the samples were injected onto an LC-oaToF-MS system. Next, the LC-MS fluxomics data were processed with an informatics pipeline that consists of data transfer, data conversion into open source standards, and import of the converted data for peak detection, curation, natural abundance correction and visualization. The analysis of the human non-small cell lung carcinoma cells flux data indicated that the lactate fractional enrichment of the 13C3 isotopologue is significantly higher in spheroid vs. monolayer cell cultures. Since 13C3-lactate is the major isotopologue produced from 13C6-glucose, the observations suggest that glycolysis is significantly upregulated in spheroid cell cultures. Moreover, key intermediates in the TCA cycle had higher 13C2 isotopologues. In turn, synthesis of pyruvate 13C3 isotopologues can be attributed to 13C6-glucose, suggesting a higher contribution of glucose to the TCA cycle via acetyl CoA in spheroid cell cultures.

P-609 Meta-analysis of tracer-biotransformation experiments of exogenic and endogenic isotopically-labeled tracer compounds with LC-HRMS and MetExtract II

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Tracer-based metabolomics studies have become more popular with the increased availability of isotopically-labeled tracers. By spiking a biological organism (e.g. plants, fungi, mammals) with these labeled tracers, virtually all biotransformation-products can be detected that are newly synthesized under the tested experimental conditions. A powerful workflow for the detection of biotransformation-products from secondary metabolism reactions of either endogenous or exogenous tracers, is the TracExtract workflow. It combines LC-HRMS with the custom-tailored software MetExtract-II for the fully automated and untargeted detection of novel biotransformation-products. In this work, the results of several such studies are summarized. Mainly 13C-labeled tracers were studied with the TracExtract workflow (e.g. the mycotoxins deoxynivalenol, T2/HT2 and zearalenone in wheat ears, tryptophan and phenylalanine in wheat and grapes, methylation artifacts during sample preparation, glucose for the detection of RNA-modifications). For example, 172 biotransformation-products of phenylalanine in wheat plants infected with Fusarium graminearum, more than 50 novel detoxification products of mycotoxins in different cereal plants, and 88 sampling artifacts of methanol were successfully detected. The number of identified metabolites varied greatly (10-172) depending on the studied tracers. No modifications of the general workflow or LC-HRMS setup were required for the majority of the performed experiments. The automated, untargeted search for novel biotransformation products was very specific in all experiments and resulted in only few (1-5) false-positives on average. The results demonstrate that isotopically-labeled tracers and the developed workflow are generic, highly specific and can be applied to virtually any isotopically-labeled tracer as there is no need for complicated experimental setups.

POSTER SESSIONS 1 AND 2 – Monday and Tuesday – all odd number posters will be on display.
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*AWARD WINNERS

TECHNOLOGY

P-610 Comparison of Methanol, Acetonitrile and Ethanol with Respect to Solvent-Induced Artifact Formation in Biological Samples

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Organic solvents play a critical role in metabolomics experiments. They are usually indispensable for extraction or separation of metabolites from biological matrices. Ideally, the solvent should behave in an inert state and in no way alter the metabolites to be analyzed. While, it has already been shown that the extraction of plant material with methanol resulted in the formation of numerous solvent-induced artifacts (Sauerschnig et al., 2017), no studies on other common solvents have been published so far. Here, we have used a similar approach to investigate and compare artifact formation by acetonitrile, ethanol and methanol. To this end, the filamentous fungus Trichoderma reesei was cultivated in synthetic minimal liquid medium and both the culture media supernatant and the fungal mycelium were extracted separately with each of the three solvents. In each case, the native and deuterated form of the solvent was used in parallel. The experiment was also performed on fully labeled 13C samples to be able to unambiguously classify metabolites to be of biological origin and on a defined standard mix. The samples were measured by LC-HRMS and the respective measurement data was then searched for coeluting feature pairs with identical peak shape and whose m/z values differed by exactly a multiple of the mass increment between 1H and 2H. Similar to Sauerschnig et al. (2017), numerous artifacts induced by methanol were found. The poster will demonstrate that ethanol- and acetonitrile-derived extracts did also contain solvent-induced artifacts, albeit to a much lesser extent than in case of methanol.

P-611 Investigating metabolic flux in central metabolic pathways associated with isocitrate dehydrogenase 1 mutations using [1,2-13C2]Glucose

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Isocitrate dehydrogenase mutations are found in a range of cancers including glioma and amyloid leukaemia and produce a change of enzyme function leading to the production of significant amounts of 2-hydroxyglutarate (2-HG), a metabolite that has now become one of the best known small molecule biomarkers in cancer. The mechanism of tumorigenesis is still not fully understood however. A number of metabolomics studies have investigated changes in metabolite profiles but few have investigated the impact of the mutation on metabolic flux. In this study [1,2-13C2] glucose was used as a tracer to quantify metabolic flux in LN18 IDH mutant (IDH1) and IDH wild type cells. Anion-chromatography coupled to a Q-exactive orbitrap MS system was used to measure glycolytic, pentose phosphate pathway and TCA cycle metabolites and concomitant isotopomer distributions. INCA was then used to calculate flux values for each metabolic transformation by minimizing the difference between the measured labelling patterns and those simulated by the metabolic model with 95% confidence intervals. LN18 mutant cells show altered flux through central metabolic pathways, a high flux from α-ketoglutarate to 2-hydroxglutrate with a decreased flux from α-ketoglutarate to succinate, fumarate and malate indicated decreased oxidation of glucose in the TCA cycle compared to LN18 wild type cells. A decreased flux through PDH and an increased flux from pyruvate to lactate through LDH suggested that IDH1 mutant cells undergo broader metabolic reprogramming than has previously been reported. It is anticipated these metabolic alterations in mutant IDH1 cells will help identify potential new therapeutic targets.

P-612 Tracer-based metabolomics for Immune Cells: Exploring Central Carbon Metabolism in Human Natural Killer Cells

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CO-AUTHORS: ***Liesbeth Vereyken, Ian Strickland, Rob J. Vreeken***

While immunotherapy is emerging as a successful alternative approach to classical drug therapy for many disease areas, such as cancer and chronic infectious diseases, still little is known about the fine-tuning between metabolism and functions of immune cells in health and disease states. Notably, central carbon metabolism, as a central metabolic pathway providing energy, cellular building blocks and precursors for cell signaling and epigenetic factors, is of key interest to understand the major metabolic routes sustaining immune cells functions. To quantify central carbon metabolites and determine the relative contribution of specific metabolic pathways (i.e. intracellular metabolic fluxes), we established a tracer-based metabolomic platform using stable isotopes together with metabolic network modeling. At first, we established in vitro immune cell line models. Specifically, human lymphoma NK-92 and leukemia KHYG-1 natural killer cells were fed with 13C6-glucose for 24h, to reach isotopic steady state prior to metabolite extraction. To monitor the major branching routes of central carbon metabolism, including glycolysis, tricarboxylic acid cycle, pentose-phosphate pathways, we developed a general analytical method for small molecules-amino acids and -organic acids, using an UPLC HILIC column system for separation coupled with a Q-Exactive HF orbitrap mass spectrometer. First experiments demonstrated incorporation of the 13C isotope label into downstream metabolites of glucose. Next, this platform will be further validated by dissecting the various metabolic modes associated with different activation or inhibitory stimuli on NK cells in vitro and finally on (pre-)clinical samples of chronic infectious diseases (e.g. Hepatitis B, HIV...).

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TECHNOLOGY

P-613 Glutamine and nitrogen metabolism in cancer

PRESENTING AUTHOR: ***Susanne Fürst, BIMSB, MDC, DKFZ, DKTK, Germany***

CO-AUTHORS: ***Safak Bayram, Guido Mastrobuoni, Christin Zasada, Stefan Kempa***

Metabolic reprogramming is a required step during oncogenesis. It is triggered by activation of oncogenes and loss of tumor suppressors and leads to an activation of central metabolic pathways to support cell growth and proliferation. Already in the 1920th Otto Warburg identified glycolysis as deregulated in cancer. These findings highlighted central metabolism as therapeutic target. Beside the activation of the glycolytic pathway also nitrogen metabolism is enhanced in order to support the de-novo biosynthesis of nucleotides as building blocks of DNA and RNA synthesis. The required nitrogen can stem from amino acids like glutamine or alanine or from free ammonium. In order to quantify the usage and activity of metabolic pathways in vitro and in vivo we have developed pulsed stable isotope resolved metabolomics (pSIRM). The applied GC-MS based technology enables the absolute quantification of metabolites and at the same time the determination of stable isotope incorporation. In a next step to quantify the dynamics of nitrogen and carbon metabolism simultaneously we are developing new methods by applying ultra high resolution mass spectrometry. With the new technology we hope to better characterize the metabolic mode of cancer cells and to identify suitable therapeutic targets.

P-614 Evaluation of intestinal functionality using tracer metabolomics

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The intestine is one of the key organs absorbing and metabolizing nutrients required for survival. Clinical phenotypes like e.g. inflammatory bowel disease, result in disturbed nutrient uptake and several health issues in patients. However, clinical tests used for monitoring intestinal functionality are either invasive, unspecific or unable to monitor the subtle changes. During inflammatory process, arachidonic acid (AA) is released through phospholipase A-2 mediated hydrolysis of epithelial phospholipids and further metabolized to prostaglandins and other eicosanoids. Therefore, we aim to establish a stable-isotope mediated metabolomics platform to monitor the function of intestine using stable isotope-labeled AA. For proof-of-concept purpose, we used Caco-2 cell model and induced its inflammatory response by interleukin-1β, tumor necrosis factor-α and interferon-γ. Furthermore, we feed Caco-2 cells with 13C-labeled AA and evaluate its incorporation into the phospholipids and its downstream metabolites. To monitor the changes of phospholipids, we have established a shotgun lipidomics platform, using nano-electrospray coupled to a Q-Exactive HF orbitrap mass spectrometer. To evaluate the release of eicosanoids, we used a targeted UPLC-MS/MS method (for 130 eicosanoids) on an API 6500 QTRAP system. Our first results confirmed uptake of C13-AA in the Caco-2 cells and release of prostaglandins into medium from cytokines-stimulated Caco-2 cells. These will also be further optimized in view of dynamics of inflammatory processes and further applied in various in-vivo systems.

P-615* Isotype-specific regulation of whole-body gluconeogenesis by AAK/AMPK in live C. elegans observed with real-time in-organism NMR metabolomics

PRESENTING AUTHOR: ***Tin Nguyen, Seoul National University, South Korea***

CO-AUTHORS: ***Yong Jin An, Jin Wook Cha, Yoon-Joo Ko, Hanee Lee, Christine H Chung, Sang-Min Jeon, Junho Lee, Sunghyouk Park***

Traditional metabolomics employs sample extraction of tissues and cells, making it difficult to understand the actual metabolic flux in a living system at the whole-organism level. Combining whole-body 13C-labeling strategy, NMR spectroscopy, and optimized sampling methods, we developed an in-organism NMR metabolomics approach for live C. elegans animals. The convenient approach allowed real-time monitoring of metabolic activity at the whole-body level of live worms with high sensitivity and resolution. When applied to C. elegans with mutations in AAK/AMPK, a metabolic master regulator, this approach revealed unique metabolic role of AAK-1 and AAK-2 isotypes in live C. elegans. Particularly, the aak-1 knockout animals exhibited enhanced glucose production under starvation, strikingly opposite to aak-2 knockout animals. Unusually high compensatory expression of the reciprocal isotypes in each mutant and the results for the double knockout suggested an unconventional phenotype-genotype relationship and the epistasis of aak-2 to aak-1 in glucose production. Mechanistically, the gene expression and 13C-isotope incorporation results showed that aak-1 KO metabotypes are due to reduced TCA and glycolysis and enhanced gluconeogenesis through the activation of fatty acid oxidation and glyoxylate shunt. Revealing isotype-specific roles of the catalytic subunits of AAK/AMPK, our approach should be readily applicable to other metabolic models involving C. elegans.

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TECHNOLOGY

P-616 Wikidata and Scholia as a hub linking metabolite knowledge

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CO-AUTHORS: *Denise Slenter, Daniel Mietchen, Chris T. Evelo, Finn Å. Nielsen*

Making chemical databases more FAIR supports identification of unexpected chemical compounds (toxicants, drug metabolites, etc) in untargeted metabolomics experiments. We here discuss Wikidata: it is a machine readable database, boosting the interoperability of metabolite databases. Thanks to the Wikidata:WikiProject Chemistry community, there is a growing amount of information about metabolites: Wikidata currently has over 150 thousand compounds with InChIKeys and 70 thousand CAS numbers. Ongoing work by this WikiProject includes capturing chemical classes and chemical compounds in the various Wikipedias as machine readable data. Other projects include covering human drugs [2], MeSH Chemicals and Drugs, and volatile organic compounds. We here introduce our contributions to the WikiProject Chemistry to support FAIR-ification of metabolite knowledge. For example, we proposed Wikidata properties to annotate compounds with database identifiers for the EPA CompTox Dashboard [2], the SPLASH [3], and MassBank. Furthermore, we used a combination of Bioclipse and QuickStatements to add missing compounds for biological pathways from WikiPathways [4]. Finally, we introduce an extension of Scholia [5], visualizing data about compounds and compound classes, including external identifiers, physicochemical properties, and an overview of the literature from which the knowledge is derived. At the time of writing, three thousand chemicals are linked to more than 170 thousand research articles. 2. Putman TE, et al. Database. 2017;2017(1). 3. Williams, AJ, et al. J. Cheminform. 2017;9:61. 4. Wohlgemuth G, et al. Nature Biotechnology. 2016;34(11):1099–101. 5. Slenter DN, et al. NAR. 2018;46:D661–D667. 6. Nielsen, FÅ, et al. The Semantic Web: ESWC 2017 Satellite Events, 2017.

P-617 Optimisation of nanoelectrospray direct infusion mass spectrometry metabolomics for 96-well based in vitro, high throughput screening

PRESENTING AUTHOR: *Julia Malinowska, University of Birmingham, United Kingdom*

CO-AUTHORS: *Julia M. Malinowska, Taina Palosaari, Jukka Sund, Donatella Carpi, Maurice Whelan, Mark R. Viant*

High throughput screening (HTS) is becoming a recognised approach for supporting decision-making in chemical safety assessment, whilst in vitro metabolomics is a promising tool to accelerate a departure from the use of animal models in toxicity testing. The objective of this study was to seek compatibility of high-resolution spectral-stitching nanoelectrospray direct infusion mass spectrometry (nanoDIMS) metabolomics (University of Birmingham) and an HTS platform (EU Reference Laboratory for Alternatives to Animal Testing). Low biomass cell samples (50,000 hepatocytes of HepaRG per well) were prepared for metabolomics analyses using a newly established automated protocol and solvent system (Biomek FXp laboratory automated workstation) and analysed using a modified nanoDIMS method (Thermo Scientific Orbitrap Elite) suitable for small sample sizes. The proposed method was assessed with respect to sensitivity and reproducibility of the entire workflow from HepaRG sampling to measurement of the metabolic phenotype. It has been demonstrated that the optimised nanoDIMS metabolomics method provides acceptable sensitivity (>3,500 non-background peaks routinely detected) as well as acceptable intra- and inter-plate variability (median relative standard deviation <30%) in both polar and nonpolar nanoDIMS assays. Baseline characterisation of HepaRG endogenous metabolism over time and a pilot study with a model toxicant (cadmium chloride) are underway, revealing changes in the metabolome through a 48-hr culture period. This newly established analytical method shows that in vitro metabolomics may be readily applied as an additional high content assay in 96-well HTS, complementing HT measures of the transcriptome and image phenotype.

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