Abstracts submitted for CMC2020

Oral Presentations - invited and confirmed

Novel approaches in clinical metabolomics: breath testing

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Breath test is a novel approach for population screening and early diagnosis of complex pathologies, such as cancer [1].

Detection of volatile organic compounds (VOCs) within exhaled breath offers an attractive new strategy for non-invasive screening, diagnosis and monitoring of disease. The numerous VOCs contained in breath potentially reflect biochemical processes acting in the whole body [2]. Breath is a complex biological matrix with VOCs at trace concentrations; techniques used to quantify these compounds must be highly sensitive and specific. Mass spectrometry nowadays is one of the most innovative techniques in analytical chemistry, in continuous evolution, which allows high specificity and sensitivity, in a short analysis time. Despite clear advantages, breath analysis is still a young field and few breath test are available in the clinical practice [3].

We are establishing a workflow for multi-centre large-scale clinical trials for biomarker discovery in different types of gastro-intestinal cancer. It involves analysis of breath samples with the use of different mass spectrometry platforms, to assure high quality results and reliability.

The first screening of patients was performed with the use of selected ion flow tube mass spectrometry (SIFT-MS) using direct sampling technique. With this technique, patients directly breathe into a heated inlet connected to the mass spectrometer, producing real time results. The second breath sample from the same patient was collected using Nalophane bags and then transferred in thermal desorption tubes (TDt) to be analysed with gas chromatography mass spectrometry (GC-MS). The complementary quantitative real-time output of SIFT-MS together with specificity of qualitative GC-MS analysis assure high quality results.

The final VOCs profiling using the two platforms will give us an introductory outline for the characterization of specific biomarkers involved in human gastric cancer.

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2. Smith, D., C. Turner, and P. Spanel, Volatile metabolites in the exhaled breath of healthy volunteers: their levels and distributions. J Breath Res, 2007. 1(1): p. 014004.

3. Hanna, G.B., et al., Accuracy and Methodologic Challenges of Volatile Organic Compound-Based Exhaled Breath Tests for Cancer Diagnosis: A Systematic Review and Meta-analysis. JAMA Oncol, 2018: p. e182815.

How to cheat with data analysis (and how not to)

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Data-mining has many nicknames such as exploratory analysis, machine learning, artificial intelligence, chemometrics etc. In this talk, we will use simple examples that will highlight that

1) if you look at one variable or measurement at the time, you're are throwing away the most important information in your data and

2) you can easily handle all the data that is relevant for your problem. You do not need to select data to simplify.

3) there are common pitfalls that can lead you to wrong conclusions when you analyze complex data

We will give several examples on how to extract all the information from your data without getting overly optimistic results.

A comprehensive metabolic atlas of atherosclerosis reveals a novel sphingolipid pathway of foam cell apoptosis. From humans, to models and back.

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Atherosclerosis remains a leading cause of mortality and morbidity. Increased plaque stenosis is associated with high risk of plaque rupture, which can lead to adverse health events. In order to map the metabolic dysregulations of plaque formation, a comprehensive metabolomics approach was employed (UHPLC-MS lipidomics, HILIC-UHPLC-MS, NMR, oxylipin profiling and DESI-MS Imaging). Plaques from 78 patients were used to compare arterial plaque to adjacent non-plaque tissue. Dysregulated molecules and pathways included: free unesterified cholesterol (FUEC), oxidized cholesteryl esters, purines, pyrimidines, sphingolipids, oxylipins and acylcarnitines. Although sphingolipids are known modulators of apoptosis in atherosclerosis, a previously unassociated sphingolipid, namely phosphatidylethanolamine-ceramide (PE-Cer), was detected with high statistical significance ($p=9.8x10-12$) and 2-fold reduction in plaques. PE-Cer also demonstrated the highest (inverse) correlation to FUEC ($\rho=-0.76$).

From in vitro validation studies, using peripheral blood monocyte-derived macrophages (MDM) ($n=7$), the model representing advanced atherosclerosis (treated with acLDL/FUEC) confirmed a 2-fold reduction of PE-Cer ($p<0.001$). This was accompanied with a reduction of the SAMD8 gene RNA (responsible for PE-Cer synthesis), along with elevated apoptosis (flow cytometry). A statistical association of PE-Cer to FUEC was further confirmed ($r=-0.80$). Comprehensive examination of the sphingolipid pathway, indicated an increase in de novo ceramide synthesis, further to the recognised sphingomyelin hydrolysis.

Studies in tissue and serum from LDLR-/- mice (n=6), verified a loss of sphingolipid homeostasis, while studies in human plasma/serum (n=614) confirmed an association between free cholesterol and sphingolipids.

Overall, the PE-Cer pathway demonstrates a potentially pivotal role in advanced atherosclerosis and specifically foam cell apoptosis. Further unrecognised sphingolipid pathway alterations and connections to FUEC, are revealed.

The urine and fecal metabolome decomposed into food, drug, host, and microbiome-related components through substructure-based approaches

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Deciphering complex metabolite mixtures like urine and fecal extracts remains a challenging task. Key reasons include the sheer number of information-rich mass fragmentation spectra modern mass spectrometers produce. Here, we use this information-richness to our advantage to decompose the urine and fecal metabolome. We use computational approaches that discover spectral similarities and mass spectral patterns corresponding to the biochemical building blocks of molecules (here termed substructures).

First, the metabolome mining tool GNPS molecular networking is applied to cluster spectra into molecular families followed by the substructure discovery tool MS2LDA to highlight the presence and absence of substructures across those families. Then, annotation tools like Network Annotation Propagation are used to collect plausible candidate structures for each molecular family.

Subsequently, the MolNetEnhancer workflow annotates molecular families with ClassyFire chemical classification ontology terms based on the collected candidate structures. Finally, we present recent progress in the introduction of MotifDB, a repository for annotated spectral patterns (Mass2Motifs) from MS2LDA. All this additional structural information eases the interpretation of large mass spectral molecular networks.

We showcase that for urine and fecal metabolome data it then becomes possible to connect molecules to their possible origins, i.e., food, drugs, the human host, or the microbiome. Moreover, we show how a drug screening approach was developed based on molecular networking of urine mass fragmentation data. In conclusion, substructure annotation is a promising avenue to overcome challenges of metabolite annotation in complex metabolite mixtures such as the urine and fecal metabolome. These developments represent key steps towards using untargeted metabolomic approaches as clinical diagnostic and predictive workflows, for example to link food or drug exposure to health advantages or risks.

A blood lipidomic and proteomic integrative approach highlights immunity and lipid pathways in Alzheimer's disease

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Introduction: There is an urgent need to understand the molecular mechanisms underlying Alzheimer's Disease (AD) to enable early diagnosis and develop effective treatments. Here we aim to investigate Alzheimer's dementia using an agnostic lipid, protein and gene multi-omics intergrative approach.

Methods: A proteomics dataset (201 AD, 104 MCI and 97 controls) from AddNeuroMed (ANM) cohort and a lipidomics dataset (185 AD, 40 MCI and 185 controls) from Dementia Case Register & ANM cohort were utilised for weighted gene co-expression network analyses (WGCNA). An additional cohort (Alzheimer's Research Trust) with matching proteomic data (94 AD, 55 MCI and 100 controls) was included for external validation. Modules created within each modality were correlated with clinical AD diagnosis, brain atrophy measures and disease progression, as well as with each other. The gene ontology enrichment over-representation analysis (ORA) was employed to examine the biological process for selected protein modules, while the annotations of lipid species for selected lipid modules were conducted. Associations between AD risk loci and lipid/protein modules that showed high correlation with AD phenotypes were also explored.

Results: The resulting 20 and 17 modules for lipid and protein networks were validated through module preservation respectively. Four lipid modules showed significant correlation with AD phenotypes. These modules consisted mainly of phospholipids and were correlated with AD risk loci involved in immune response and lipid metabolism. The ORA for selected five protein modules associated with AD phenotypes highlighted proteins involvement in immune response.

Conclusions: In this preliminary study, we provided a pipeline for conducting multi-omics study. In addition, these network analyses highlighted the role of immunity and lipid pathways in AD.

Poster Presentations - invited and confirmed (more to come!)

Plasma metabolomics identifies markers of kidney function in 3,089 Europeans with type 2 diabetes

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Background and aims: Chronic kidney disease reduces adult lifespan significantly and is a major cause of cardiovascular and end stage renal disease. It occurs in 40% of diabetics. Our aim was to identify plasma metabolites associated with kidney function and albuminuria among people with type 2 diabetes.

Methods: This study included five independent Dutch cohorts, (a. The Hoorn Diabetes Care System (n=998) cohort, b. The Maastricht Study (n=848), c. The Rotterdam Study (n=426), d. The Netherlands Epidemiology of Obesity Study (n=675) and e. The Cohort of Diabetes and Atherosclerosis Maastricht study (n=145)) totaling upto 3,089 people with type 2 diabetes. The participants had a mean estimated glomerular filtration rate (eGFR) of 83.5 (ml/min/1.73m2) at a mean age of 61.9 years.

Linear regression based cross sectional association was tested between single plasma metabolites (n=149) and eGFR and urinary albumin-to-creatinine ratio (UACR) in each study. Covariate adjustments included age, sex, systolic blood pressure, body mass index, medication (lipid-modifying, glucose-lowering, anti-hypertensive), smoking, diabetes duration, HbA1c, and eGFR or UACR, where appropriate. An inverse variance random effect meta-analysis was performed.

Results: After adjustment for multiple testing, 125 metabolites associated significantly with eGFR while none with UACR. Inverse associations were demonstrated for creatinine, amino acids and glycoprotein acetyls and fatty acids (Omega-6, Linoleic acid, and PUFA; PFDR<1×10-4). Glycoprotein and small and medium VLDL were associated with UACR levels (P<0.01) before multiple testing correction, but not after.

Conclusion: The findings suggest that serum lipoprotein Subclasses of HDL, and ApoA1 may be associated with an improved, while amino acids and fatty acids like Omega-6 and Linoleic acid with a reduced kidney function.

SWATH Acquisition for Untargeted Metabolomics and Comprehensive Metabolite Quantitation

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The field of metabolomics and metabolic profiling faces a large challenge, in accurately identifying and quantifying hundreds to thousands of metabolites in a single run. Generally, quantitative metabolomics is performed on triple quadrupole or QTRAP® systems in a targeted manner by multiple reaction monitoring (MRM) for enhanced sensitivity and selectivity. Internal standards are often used to enhance quantitative accuracy. SWATH acquisition, a data independent acquisition (DIA) technique, is well adopted in quantitative proteomics, but still not commonly used in quantitative profiling of metabolome.

SWATH acquisition has been shown to identify a higher number of metabolites compared to the traditional data dependent acquisition (DDA) approach, thus enabling broader metabolome coverage. Here, SWATH acquisition is used for quantitation of selected metabolites using the MS/MS data, for reduced interferences, improved signal-to-noise and deeper metabolite quantitation. The use of MS/MS fragments for metabolite quantitation provides better selectivity, and ultimately increased sensitivity compared to simply relying on the precursor ion for quantitation. A SWATH acquisition map contains MS and MS/MS information of every detectable metabolite in the sample and is therefore a digital archive of the sample. This reduces the need to go back and re-run samples; data can just be re-mined as the hypothesis evolves.

Automated annotation of untargeted LC-MS metabolomics features using all-ion LC-MS/MS and metabolite fragmentation data

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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. Even for known compounds, the annotation of such features remains a bottleneck, requiring additional MS/MS experiments and expert knowledge of molecular fragmentation [1]. 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 window experiments (MSE or MSALL) which generate spectra composed of fragments from multiple parent ions. Herein, we developed an automated strategy to annotate LC-MS datasets using all-ion fragmentation data, by combining correlation-based parent-fragment ion reconstruction with known molecules fragment matching. The annotation pipeline is available as an R package, MetaboAnnotatoR. This strategy enabled the correct annotation of several hundred features from XCMS outputs [3] in a set of human serum samples, with results comparable to those obtained by another LC-MS annotation tool, MS-DIAL [4]. In comparison to MS-DIAL, MetaboAnnotatoR has the advantage of working on targeted annotation of user-defined features lists, e.g. statistical significant features, which may result from isotopologues, adducts and fragments. In summary, the proposed annotation strategy improves the overall analysis and interpretability of untargeted LC-MS metabolomics data.

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LipidomeR – A Tool for Systematic Visualization and Interpretation of the Lipidome

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Introduction: Lipidomics is currently one of the fastest-growing areas of molecular profiling in medicine. While increasing amounts of lipidomics data are being generated, tools for analyzing, interpreting and communicating these data are lagging behind this trend. We present the lipidomeR -- a novel tool that is specifically designed for systematic interpretation of large lipidome-wide studies. The lipidomeR knits together rigorous statistical analysis and high-dimensional visualization, providing a pipeline for rapid interpretation of lipidomics studies via integrative publication-ready figures.

Methods: The lipidomeR package was developed for R and will be available through the Comprehensive R Archive (CRAN). We demonstrate the lipidomeR by analyzing publicly available lipidomics data sets from the Metabolomics Workbench repository. In three case studies, we create an integrative visualization of the lipidome in a human plasma reference sample, visualize the aberrated lipidome in breast cancer tissue, and assess the stages of progression in non-alcoholic fatty liver disease (NAFLD).

Results: First, concentrations of 403 lipid species in human plasma reference material were visualized, illustrating the diversity of concentrations in 23 classes of circulating lipids. Second, 409 lipid species from 118 tissue samples of breast tumors were visualized, revealing a pattern of cancer-driven aberrations in 12 classes of lipids. Third, 316 lipids from 88 liver tissue samples were analyzed, uncovering a lipidomic trajectory of disease progression in 7 lipid classes over the three stages of NAFLD.

Conclusion: We demonstrated that lipidomeR uncovers new insights into complex lipidomic patterns by presenting lipid levels with a systematic and integrative method. We argue that the lipidomeR approach can improve interpretability, comparability and reproducibility of lipidomics studies. To support this, we provide these case studies as reproducible examples in the package.

Mucosal metabolomic and lipidomic signature of Ulcerative Colitis

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Inflammatory bowel disease (IBD) is a chronic, relapsing inflammatory disorder in the gastrointestinal tract that affects up to 3 million people across Europe. The major forms of IBD, Ulcerative Colitis (UC) and Crohn's Disease (CD), are characterized by a dysregulated mucosal immune response triggered by several genetic and environmental factors in the context of host-microbe interaction. This overwhelming complexity makes IBD ideal for metabolomic and lipidomic studies to unravel the disease pathobiology and to improve the patient stratification strategies toward personalized medicine.

Colon mucosa biopsies were collected from 22 treatment-naive UC patients (inflamed mucosa), 15 UC patients in deep remission, and 15 healthy subjects. Metabolomic analysis was performed by combining GC-TOF-MS and UPLC-TOF-MS. In addition, lipidomic analysis was performed by means of UHPLC-QTOF-MS. In total, 177 metabolites from 50 metabolic pathways and 220 lipids from 11 different classes were quantified.

Multivariate analysis revealed a distinct lipidome and metabolome profile for each of the study groups. The most prominent changes among the study groups were in phospholipids, sphingosines, amino acids and acyl carnitine profiles. In addition, levels of long chain fatty acid ceramides were low in healed mucosa, and increased in a stepwise manner in UC remission and treatment-naïve UC patients. Pathway analysis revealed perturbation in amino acid metabolism pathways (such as tryptophan metabolism) and antioxidant defense pathway (glutathione pathway). Furthermore, our data revealed a disturbance in the long and short chain fatty acid metabolism, namely linoleic acid metabolism and butyrate metabolism.

The mucosal lipidomic and metabolomic signature in active UC reflected the homeostatic disturbance in the gut. This highlights the importance of integrating IBD-omes compartments by system biology approaches to identify key drivers of pathogenesis, and prognostic biomarkers.

A community-based study identifying metabolic biomarkers of MCI and AD using artificial intelligence and machine learning

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Background: Currently, there is no objective, clinically available tool for the accurate diagnosis of Alzheimer's disease (AD). There is a pressing need for a novel, minimally invasive, cost friendly and easily accessible tool to diagnose AD, assess disease severity, and prognosticate course. Metabolomics is a promising tool for discovery of new, biologically, and clinically relevant biomarkers for AD detection and classification.

Methods: Using a community-based sample cohort acquired from different sites across the US, we adopted an approach combining Proton Nuclear Magnetic Resonance Spectroscopy (1H NMR), Liquid Chromatography coupled with Mass Spectrometry (LC-MS) and various machine learning statistical approaches to identify a biomarker panel capable of identifying those patients with AD and mild cognitive impairment (MCI) from healthy controls.

Results: Of the 212 measured metabolites, 5 were identified as optimal to discriminate between controls, and individuals with MCI or AD. Our models performed with AUC values in the range of 0.72-0.76, with the sensitivity and specificity values ranging from 0.75-0.85 and 0.69-0.81, respectively. Univariate and pathway analysis identified lipid metabolism as the most perturbed biochemical pathway in MCI and AD.

Conclusion: A comprehensive method of acquiring metabolomics data, coupled with machine learning techniques, has identified a strong panel of diagnostic biomarkers capable of identifying individuals with MCI and AD. Further, our data confirm what other groups have reported in that lipid metabolism is significantly perturbed in those individuals suffering with dementia. This work may provide additional insight into AD pathogenesis and encourage more in-depth analysis of the AD lipidome.

Treatment of HFD induced diabetes mellitus by Metformin and natural products in rats

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Background & Aim: Asphodelus microcarpus (AM) is widely distributed over the coastal Mediterranean region, traditionally used in the treatment of diabetic conditions. The aim of the present investigation was to evaluate the antioxidant and anti-diabetic activity of the exogenous metabolites extracted from the plant.

Methods: Ethyl acetate of Asphodelus microcarpus tubers was used for this study. Diabetes was induced in rats by HFD feeding for 10 weeks. The rats were divided into following groups: Group-I: Normal control, Group-II (Vehicle): Diabetic control, Group-III: Diabetic rats (AM 10 mg/kg), GroupIV: Diabetic rats (AM 10 mg/kg+MET 100 mg/kg), Group-V: Diabetic rats (AM 20 mg/kg), Group-VI: Diabetic rats (AM 20 mg/kg+MET 100 mg/kg), and Group-VII: Diabetic rats (MET 100 mg/kg). Bodyweight of each rat in the different groups was recorded daily. Biochemical and antioxidant parameters were determined on day 21.

Results: The exogenous metabolites of AM showed a better glucose utilization and insulin resistance improvement. Oral treatment of different doses of AM extract alone and/or with metformin decreased the level of serum glucose, activity of liver alpha glucosidase, activity of pancreatic alpha amylase, MDA, CRP and leptin. Treatment showed increased level of plasma insulin, Catalase, glutathione peroxidase, liver GSH, total antioxidant capacity. HFD induced diabetic rats treated with different doses of AM extract and metformin significantly increased muscle glucose transporter 4 (GLUT4), also showed a remarked regenerative effect on the liver, kidney and pancreas.

Conclusion: The antioxidant, anti-inflammatory and anti-diabetic effect of the exogenous metabolites extracted from Asphodelus microcarpus suggests a potential therapeutic treatment for diabetes. Further study is required to determine the pharmacological effects of this natural compounds using metabolomics technology.

Linking Altered Tumour Metabolism to Therapeutic Targeting – A Multi-Pronged Approach

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Preclinical to clinical translation of anticancer therapies is encumbered by heterogeneity. This is particularly evident in the context of tumour metabolism; altered metabolism is a long-known hallmark of tumours, however targeting is encumbered by inter- and intratumoral differences which current clinical practices fail to recognise.

In the present work we seek to address this shortcoming. In ovarian cancer, we have developed an ex vivo organotypic slice culture platform to rapidly assess the efficacy of anticancer therapies in a patient-specific timeframe. We have validated our approach for its ability to preserve nascent patient characteristics, retain viability, recapitulate the tumour metabolic landscape, and assess therapeutic response. We are using this

platform to give new life to previously trialled inhibitors of tumour glycolysis, oxidative phosphorylation, fatty acid oxidation, and glutaminolysis, to assess their antitumour efficacy in a patient-specific setting, importantly in a short enough timeframe to provide clinical utility. To our approach we have also integrated the use of MALDI mass spectrometry to spatially delineate metabolic subpopulations of cells in patient tumours in regards to their chemotherapeutic response and their response to antimetabolites. We are coupling this to microscopy and histology approaches to concurrently define tumour subpopulations in terms of oncogene expression and metabolic signature, and relate this to their specific therapeutic response. It is hoped that the present work can contribute to bridging the gap between tumour metabolic signatures and their successful targeting.

Metabolic effects of gastric bypass surgery - is it all about calories?

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Bariatric surgery is an efficient and rapid method to induce weight loss, with the added benefit of also frequently resulting in remission of type 2 diabetes (T2D). Unpaired studies have shown bariatric surgery and dietary interventions to impact differentially on multiple hormonal and metabolic parameters, suggesting that bariatric surgery causes T2D remission at least partially via unique mechanisms.

In the present study, extensive metabolite profiling was conducted in patients with (n=10) and without T2D (n=9) subjected to Roux-en-Y gastric bypass surgery (RYGB). Analyses were conducted in blood samples collected during a mixed meal test at baseline, after the pre-surgical very low-calorie diet (VLCD) intervention, immediately after RYGB, and after a 6 weeks recovery period. Thereby, we could compare fasted and post-prandial metabolic consequences of RYGB with those elicited by VLCD in the same patients.

VLCD resulted in a pronounced increase in acylcarnitine levels, whereas RYGB, both immediately and after a 6-weeks recovery period, resulted in a smaller, but opposite effect. Furthermore, we observed profound changes in lipid metabolism, especially lipid species containing unsaturated fatty acyl chains, following VLCD, but not in response to RYGB.

In conclusion, most changes previously associated with RYGB were found to rather be consequences of the pre-surgical dietary intervention. Overall, our results question previous findings of unique metabolic effects of RYGB and suggest that the effect of RYGB on the metabolite profile is mainly attributed to caloric restriction.

Targeted Metabolomics Analysis: High-Throughput Biomass Primary Metabolite Profiling by Liquid Chromatography in Tandem with MS/MS Method

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Computational biology representations of metabolism often include cellular growth reactions requiring knowledge of biomass composition for accurate predictions [1]. The key challenges for researchers include the identification and quantitation of diverse metabolites in complex biological systems as well as capturing changes in a reproducible manner that is representative of the events that take place in vivo. Thus, further developments are essential for understanding changes that occur in microbial communities (eg. gut microbiota) under different conditions. This highlights the importance of developing an efficient high-throughput method with the optimal metabolome coverage and assurance of valid and accurate results relevant to computational biology applications.

One analytical challenge for the simultaneous analysis of multiple metabolites is their different physicochemical properties [2]. Thus, there is no universal technique that can be used to assess the entire metabolome: while RPLC can be used for profiling of mid-polar and non-polar metabolites, the separation of highly hydrophilic central carbon metabolites leaves room for improvement on the instrumentation side [3].

In this approach, we group the compounds that are suitable for simultaneous analysis and optimize the instrument conditions as well as the sample preparation, accordingly. The research is directed towards the development and validation of LC-MS/MS method for the simultaneous estimation of metabolites that are directly involved in cell growth, development, and reproduction. For the optimal metabolome coverage and annotation, LC-MS/MS method operating in MRM detection mode is used. The major consideration is given to the column and gradient selection, QC samples, sample preparation methods, the matrix effects, and other parameters required to achieve the desired separation. The proposed approach represents a time-efficient and accurate method for diverse applications in various fields such as medical, environmental, nutrition, and agricultural sciences.

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Showcasing Longitudinal Metabolomic Datasets: Accessible, Fast and Simple

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The increasing number of longitudinal metabolomics studies enable time-resolved exploration of individual molecular changes over days, weeks, or even years, as well as insights into metabolic responses to specific perturbations. Adding the dimension of time significantly increases the complexity of metabolomics data. For

an in-depth exploration of these time-resolved datasets, tools for comprehensive presentation and visualization of data and statistical results are crucial.

To this end, we are developing a reusable computational framework, which integrates a pipeline covering the steps from time-course data pre-processing to interactive visualization of results. This R-based framework enables efficient data exploration of time-resolved metabolic features for non-data scientists and can be easily customized to any longitudinal metabolomics study. The output is depicted within an intuitive web-based graphical user interface, which can be accessed through any browser. Our framework combines multiple modules to visualize data and statistical results. Within our advanced search module, the user can discover metabolites of their interest or find similar metabolic temporal patterns. Further modules facilitate a quick interactive visualization of molecular dynamics by time course plots, as well as providing a systems overview of metabolic changes in dynamic networks. An example use case of our framework can be found at http://humet.helmholtz-muenchen.de/.

A network-based, multi-omics integration framework for target prioritization in Alzheimer's disease

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Alzheimer's disease is an untreatable, neurodegenerative disorder and the leading cause of dementia. Growing evidence implicates various metabolic pathways in the pathophysiology of AD including membrane lipid dysregulation, impaired glucose uptake and altered cell signalling. Large-scale metabolomics studies provide tools to pinpoint critical biochemical failures linked to the disease. However, to elaborate on potential underlying molecular pathomechanisms and, ultimately, to identify novel potential therapeutic targets, it is crucial to view metabolic readouts in a multi-omics context.

Here, we propose a network-based, multi-omics framework developed with the graph. database Neo4j that allows the large-scale integration and analysis of data on biological entities across omics, as well as results from association analysis with specific AD (endo)phenotypes. The backbone of this framework comes from known biological relationships such as gene-transcript-protein relations and functional/pathway annotations available in public databases. This backbone is augmented with experimental, quantitative data across omics (e.g. eQTLs or mQTLs) derived in population-based studies. To identify modules within this network that are potentially relevant to AD, we extend the framework using large-scale association data for AD (e.g. MWASs or GWASs with AD and its biomarkers). The resulting network is comprised of >60 million nodes (biological entities), representing >30 different data types and >500 million edges (relationships between entities).

We mined this comprehensive, multi-scale network of biological information using established graph algorithms to identify potentially disease-related modules of tightly interlinked entities, and were able to obtain several subnetworks significantly enriched for AD-associations. Further applications will include the search for druggable regulators of metabolism through metabolomics driven hypothesis and the prioritization of potential targets.

Integrating genetics with newborn metabolomics in Infantile Hypertrophic Pyloric Stenosis

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Infantile hypertrophic pyloric stenosis (IHPS) is a serious disease in newborns caused by hypertrophy of the pyloric sphincter smooth muscle. Its etiology is incompletely understood, but involves genetic predisposition and environmental risk factors. We previously performed a series of genome-wide association studies (GWAS) of IHPS (Feenstra et al. Nat Genet 2012; Feenstra et al. JAMA 2013; Fadista et al. Hum. Mol. Genet. 2019) identifying a number of associated loci, including genetic associations between lipid metabolism and IHPS risk.

Building on that finding, the present study aimed to investigate associations between a wide array of lipid-centric metabolites in newborns and IHPS. Using a matched case-control design and the Biocrates p400 kit, we found that newborns who later developed IHPS had lower levels of phosphatidylcholines (PC(38:4) and several others), histidine and short-chain acylcarnitine AC(2:0) compared to control children. Notably, the associations were driven by case-control pairs with a later age at sampling.

Several lines of evidence suggest that these results reflect different feeding patterns after birth. First, metabolites levels in the first days after birth are much less influenced by feeding than later, when enteral nutrition has been fully established. Second, bottle feeding is a well-known IHPS risk factor, and in our data more IHPS cases than controls had a diagnosis for neonatal difficulty in feeding at breast in the Danish National Patient Register. Third, integrating the metabolomics data with GWAS data, we found that genetic variants known to be associated with levels of the top metabolite, PC(38:4), did not associate with IHPS. Finally, PC(38:4) is known to be at higher levels in breast-fed infants compared to bottle-fed infants.

This study enhances our molecular level understanding of IHPS and may inform studies to unravel causal relationships between genetics and early feeding patterns and consequent progression to IHPS.

Treatment of HFD induced diabetes mellitus by Metformin and natural products in rats

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Background & Aim: Asphodelus microcarpus (AM) is widely distributed over the coastal Mediterranean region, traditionally used in the treatment of diabetic conditions. The aim of the present investigation was to evaluate the antioxidant and anti-diabetic activity of the exogenous metabolites extracted from the plant.

Methods: Ethyl acetate of Asphodelus microcarpus tubers was used for this study. Diabetes was induced in rats by HFD feeding for 10 weeks. The rats were divided into following groups: Group-I: Normal control, Group-II (Vehicle): Diabetic control, Group-III: Diabetic rats (AM 10 mg/kg), Group-IV: Diabetic rats (AM 10 mg/kg+MET 100 mg/kg), Group-V: Diabetic rats (AM 20 mg/kg), Group-VI: Diabetic rats (AM 20 mg/kg+MET 100 mg/kg), and Group-VII: Diabetic rats (MET 100 mg/kg). Bodyweight of each rat in the different groups was recorded daily. Biochemical and antioxidant parameters were determined on day 21.

Results: The exogenous metabolites of AM showed a better glucose utilization and insulin resistance improvement. Oral treatment of different doses of AM extract alone and/or with metformin decreased the level of serum glucose, activity of liver alpha glucosidase, activity of pancreatic alpha amylase, MDA, CRP and leptin. Treatment showed increased level of plasma insulin, Catalase, glutathione peroxidase, liver GSH, total antioxidant capacity. HFD induced diabetic rats treated with different doses of AM extract and metformin significantly increased muscle glucose transporter 4 (GLUT4), also showed a remarked regenerative effect on the liver, kidney and pancreas.

Conclusion: The antioxidant, anti-inflammatory and anti-diabetic effect of the exogenous metabolites extracted from Asphodelus microcarpus suggests a potential therapeutic treatment for diabetes. Further study is required to determine the pharmacological effects of this natural compounds using metabolomics technology.

Linking Altered Tumour Metabolism to Therapeutic Targeting – A Multi-Pronged Approach

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Preclinical to clinical translation of anticancer therapies is encumbered by heterogeneity. This is particularly evident in the context of tumour metabolism; altered metabolism is a long-known hallmark of tumours, however targeting is encumbered by inter- and intratumoral differences which current clinical practices fail to recognise.

In the present work we seek to address this shortcoming. In ovarian cancer, we have developed an ex vivo organotypic slice culture platform to rapidly assess the efficacy of anticancer therapies in a patient-specific timeframe. We have validated our approach for its ability to preserve nascent patient characteristics, retain viability, recapitulate the tumour metabolic landscape, and assess therapeutic response. We are using this

platform to give new life to previously trialled inhibitors of tumour glycolysis, oxidative phosphorylation, fatty acid oxidation, and glutaminolysis, to assess their antitumour efficacy in a patient-specific setting, importantly in a short enough timeframe to provide clinical utility. To our approach we have also integrated the use of MALDI mass spectrometry to spatially delineate metabolic subpopulations of cells in patient tumours in regards to their chemotherapeutic response and their response to antimetabolites. We are coupling this to microscopy and histology approaches to concurrently define tumour subpopulations in terms of oncogene expression and metabolic signature, and relate this to their specific therapeutic response. It is hoped that the present work can contribute to bridging the gap between tumour metabolic signatures and their successful targeting.

Metabolomics - based machine learning method for the discovery and characterisation of biomarkers implicated in Non-Alcoholic Fatty Liver Disease

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Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide. However, the diagnostic approaches for NAFLD detection is challenging due to the limited availability of non-invasive biomarkers. Metabolomics coupled to machine learning can pave way to identify diagnostic biomarkers, understand disease mechanisms, and evaluate the treatment of various diseases.

Here, targeted metabolomics was performed by liquid chromatography – mass spectrometry on healthy adults and those with NAFLD. 6 machine learning approaches were applied to the metabolomics dataset – Artificial Neural Network (ANN), K- Nearest Neighbour (KNN), Logistic regression, Support Vector Machine (SVM), Decision tree and Ensemble. These were randomly split into training, validation and test sets, and included dimension reduction, feature selection, and classification model development. The accuracies of these 6 models were tested. ANN pattern recognition model has the highest area under the curve (AOC) in classifying the subjects with and without NAFLD.

The study demonstrates the potential of ANN for NAFLD metabolomics data classification in realistic situations. Further model development and independent validation testing in other cohorts are warranted.

Deep Urinary Volatile Organic Compound Profiling with Headspace Sorptive Extraction and (GCx)GC-MS for Oesophago-Gastric Cancer diagnosis

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There is a pressing need to develop new non-invasive screening tests for oesophago-gastric cancer due to its high prevalence and poor survival. Previous studies have reported that urinary volatile organic compounds (VOCs) reflect human pathophysiological status. GC-MS based methods are the main approaches for urinary VOC profiling. However, biomarker discovery is limited by often inefficient and labour-intensive solvent

extractions, by chromatographic resolution and by the unavailability of a complete and high-throughput data-preprocessing methodology for large-scale untargeted VOC analysis of urine samples.

Novel HiSorb sorptive extraction and conventional solid phase microextraction (SPME) are both tested and evaluated. GC and GCxGC combined with TOF-MS are employed and offer outstanding throughput and identification capabilities, respectively. By coupling HiSorb/SPME with (GCx)GC-TOF-MS, this project aims to discover new predictive biomarkers for OG cancer. Optimum extraction conditions are explored for HiSorb and SPME. Osmolality is measured for urine dilution correction; and VOCs are extracted using both techniques.The use of TOF allows the quantification of a selected set of potential biomarkers (aldehydes, ketones, alkanes and short chain fatty acids) while it acquires data in full scan mode, thus combining the advantages of untargeted and targeted approaches without compromising sensitivity.

A complete data pre-processing pipeline is developed, from sample aliquoting/quality control to batch correction/biological interpretation. HiSorb shows potential advantages compared to SPME, including lower fragility, better reproducibility and VOC extraction. Osmolarity normalisation corrects the influence of urine dilution. Reproducibility, blank levels, instrument drift, run order and batch effects are estimated and corrected. Finally, the applicability of the method is tested in a pilot cohort of 70 patients, showing different volatolomic profiles.

Development of live-cell Fourier transform infrared (FTIR) spectroscopy as a novel bioanalytical tool for diabetes/metabolism research.

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Background: Hyperglycaemia is responsible for around 4 million mortality worldwide[1]. New analysis has shown that with a new case of type 2 diabetes is diagnosed every three minutes in England and Wales[2]. There is an urgent need to develop better, more effective and innovative anti-diabetic medications. Current diabetes/metabolism research heavily reliant on the analysis of metabolite using bioanalytical tools to understand the biochemistry of diabetic cells. Due to the complexities of metabolic changes in cells, many advanced biochemical assays are needed to interrogate the cellular metabolism, which are costly, laborious and time-consuming. Fourier-Transform Infrared (FTIR) spectroscopy is a non-destructive, label-free, sensitive and high-throughput technique that has recently found to be suitable for studying living cells. FTIR have shown to be able to distinguish the stage of cell cycle[3], drug-cell sensitivity and drug resistance[4, 5], diagnose diseases[6], identify biomedical change from the clinical biomarkers in various type of cancers[7, 8,9,10].

Aims: To demonstrate live-cell FTIR can be applied to study glucose metabolism in cells exposed to normal and hyperglycaemic conditions.

Methods: Human hepatocyte carcinoma cell (HEPG2) suspension with $2x10^6$/2 mL in DMEM CO2 medium were seeded on the FTIR trough plate and were measured in attenuated total reflectance (ATR) measurement mode with ZnS as the ATR element. The seeded HEPG2 had been examined at 24, 48 and 72 hours after treatment of either normal glucose (5 mM; control) or high glucose concentration (25 mM) in DMEM CO2 medium. Difference spectrum at time 0 (immediately after adding the solutions containing high or low glucose) and spectra at the selected time point were truncated to 1440-900 cm^{-1}, followed by concave rubber band baseline correction and vector normalization before subtracted from time 0. Principal component

analysis (PCA) was used as the statistical tool to highlight any possible correlated changes observation from the experiment[11].

Results: The three replicated FTIR spectra of live cell treated in normal and high glucose medium has shown reproducible changes and are distinguishable at the time of 24th, 48th and 72nd hour after treatment. All different time points can be clearly distinguished by the first principle component (PC1). The high glucose treated cells has seen an increased the absorbance at 1088, 1240 and 1400 cm^{-1} (here indicate which spectral peaks were observed). These spectral peaks are associated with phosphate symmetric and asymmetric stretching mode (PO^{2-}) from phospholipids and DNA backbone vibration and symmetric stretching of COO^{-} from fatty acids, amino acids, lipids and carbohydrate metabolites vibrations. All high glucose treated cell at all time points has also shown a significant changes in the 1000-1200 cm^{-1} region, which is linked to change in the carbohydrate and phosphate composition within the cell.

Conclusion: FTIR live cell can detect the effect of high glucose on hepatocyte and highlighted significant changes in the carbohydrate/phosphate metabolite composition in the living cells. It can be established that FTIR can be used as the platform of diabetes glucose metabolism for drug-cell study.
Future Work: The effect of glucokinase activators (PF-04991532,100 μM), which is a novel clinical trials will be compared with Metformin (2mM, first-line diabetes drug). These two different pharmacological drugs have been reported by conventional biochemical analysis to have an opposite mechanistic effect on the cell resulting in producing different metabolites in high glucose condition (25 mM).[12] The results will confirm that live cell FTIR method can be further develop as an alternative bioanalytical tool for drug-cell metabolite studies.

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