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Centre Hospitalier Universitaire Vaudois (CHUV)

Auditoire Charlotte Olivier

Rue du Bugnon 46

1011 Lausanne

MedLem17 meeting, 23rd November 2017

DISEASE BIOMARKERS: From FUNDAMENTAL RESEARCH to POPULATION BASED-STUDIES

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Organizing & scientific committee:

Dr Olivier Gaide, Dr Maxime Pellegrin, Prof. Aurélien Thomas and Dr Natacha Turck

Scientific Program

09.00 – 09.30	Registration & welcome coffee
09.30 – 09.45	Welcome to the MedLem Dr Olivier Gaide
09.45 – 10.15	<u>Keynote 1</u> <i>"Molecular classification of tumors: A step towards personalized medicine?... Omic's technique in pathology ..."</i> Prof. Laura Rubbia-Brandt
10.15 – 11.15	<u>Elevator Pitches Session</u> <ol style="list-style-type: none">1. "GWAS genes whose expression is implicated by Mendelian randomization are highly connected in tissue-specific regulatory circuits" E. Porcu2. "CD141/CD123/DC-SIGN Monocyte-derived DC can be identified in BAL fluid from patients with different inflammatory conditions" C. Obregon3. "Impact of CYP2D6 Functional Allelic Variations on Drug-Drug Interactions involving CYP2D6" F. Storelli4. "Blood steroidomics as a tool to highlight novel biomarkers for the athlete biological passport steroide module" F. Ponzetto5. "Bayesian Genome-Wide Association Study to discover novel lifespan-associated loci" N. Mounier
11.15 – 11.45	<u>Keynote 2</u> <i>"Can we anticipate the risk of developing diabetes?"</i> Prof. Gérard Waeber



11.45 – 13.30	Poster Session & Lunch
13.30 – 15.15	<u>Oral Session</u> <ol style="list-style-type: none"> 1. <i>“Proteogenomic interrogation of human tears using ProQuantum™ High Sensitivity Immunoassay could revolutionize precision medicine in the clinic”</i> S. Gadher (Thermo Fisher Scientific) 2. <i>“Autologous blood transfusion in sports : emerging biomarkers”</i> N. Leuenberger 3. <i>“A metabolomics approach to unravel altered systemic and brain energy metabolism in Alzheimer’s disease”</i> V. van der Velpen 4. <i>“The differential plasma proteome of obese and overweight individuals undergoing nutritional weight loss and maintenance”</i> O. Cominetti
15.15 – 15.45	<u>Keynote 3</u> <i>“The ageing-brain cognitive diseases and their « bio »-markers”</i> Prof. Jean-François Démonet
15.45 – 16.00	Poster & Pitch Presentation Award Ceremony
16.00 – 16.30	Concluding message & goodbye drink



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Talks abstracts

GWAS genes whose expression is implicated by Mendelian randomization are highly connected in tissue-specific regulatory circuits

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CHUV², University of Lausanne¹

Genome-Wide association studies (GWAS) have identified thousands of variants associated with complex traits but both the causative genes and pathways often remain unknown. Most of these variants fall into regulatory regions and overlap with expression Quantitative Trait Loci (eQTLs), indicating their potential involvement in regulation of expression.

We propose an advanced summary statistics-based Mendelian Randomization (MR) approach that uses simultaneously multiple SNPs and gene expression traits as instruments and exposures, respectively.

Having access to eQTLs from multiple tissues enabled us to compare the resulting causal genes for 42 complex traits across tissues.

Among our results we find examples of causally associated genes with a) overestimated effect in single-gene analysis; b) insignificant association via single-gene analysis; c) tissue-specific causal effects; d) no genome-wide significant SNP nearby in previous GWAS analysis. These results suggest that a multi-SNP and multiple-gene approach verifies MR assumptions rigorously and is a powerful test, which can reveal new insights into the tissue-specific transcriptional regulation of complex traits.

In order to translate our findings into a functional understanding of disease processes, we applied tissue-specific network analysis using ~400 tissue-specific gene regulatory circuits. We show how we find stronger connectivity enrichment when using our causally associated gene-set rather than the genes scored by physical proximity to GWAS hits.

Our results demonstrate the importance of integrating expression data from various tissues when trying to interpret GWAS results and with the increasing number of samples and tissues, our MR approach should become a standard tool in the interpretation of GWAS results.



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CD141/CD123/DC-SIGN Monocyte-derived DC can be identified in BAL fluid from patients with different inflammatory conditions.

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Monocyte-derived dendritic cells (ModDCs) have been recognized as key initiators of inflammatory responses. Little is known, however, about human ModDCs. In the present study, we characterize the CD141/CD123/DC-SIGN-ModDC in bronchoalveolar lavage fluid (BALF) from patients with different inflammatory conditions.

BALF samples were obtained from patients undergoing bronchoscopy for medical reasons. In total, 4 BALF samples from patients with adenocarcinoma, 9 with sarcoidosis, 7 with interstitial pneumonia and 7 from stable lung transplant recipients (LTR) were analysed. PBMC were analysed as control. Cells were stained using a combination of phenotypic markers found on monocytes and-DCs such as HLA-DR/CD11b/CD14/ CD141/CD123/CD1c/DC-SIGN. Cells were analysed by flow cytometry analysis (FACS).

According to FACS analysis the frequency of HLA-DR+ cells found in BALF was 2% while the frequency of HLA-DR+ cells in PBMC was 20%. However, in all fluids analysed 70% of the HLA-DR+ cells expressed CD14+/CD11b+, suggesting that the majority of HLA-DR+ cells derive from monocytes. Interestingly, we observed that the CD141/CD123/DC-SIGN triple-positive population was significantly increased in patients with pulmonary sarcoidosis and LTR, compared to patients with adenocarcinoma in which no inflammation was found.

Overall these results highlight the specific involvement of the CD141/CD123/DC-SIGN-ModDC during inflammation and suggest a possible role in the pathogenesis of sarcoidosis. Furthermore, this population was increased in LTR suggesting a role in the allogeneic graft, thus the eventual implication in cellular allograft rejection will be further evaluated. Analysis of CD141/CD1c/DC-SIGN population in BALF may be considered as a potential biomarker for inflammation including lung recipients in stable condition.



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Impact of CYP2D6 Functional Allelic Variations on Drug-Drug Interactions involving CYP2D6

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Introduction: CYP2D6 phenotypes of carriers of two fully-functional alleles (genetic activity score 2, gAS2) and carriers of one fully-functional and one non-functional allele (genetic activity score 1, gAS1) are predicted Extensive Metabolizers. However, drug-drug interactions (DDIs) might be underestimated among gAS1 patients. This study explores the impact of functional allelic variations on phenoconversion and the magnitude of DDIs among gEMs.

Method: Seventeen homozygous carriers of two fully-functional alleles and seventeen heterozygous carriers of one fully-functional and one non-functional allele participated in this trial. Dextromethorphan 5 mg and tramadol 10 mg were given at each of the three study sessions. CYP2D6 was inhibited by duloxetine 60mg (session 2) and paroxetine 20mg (session 3). The pharmacokinetics of drugs and metabolites as well as the 10-h urinary dextromethorphan/dextrorphan metabolic ratio, a validated phenotyping metrics for CYP2D6, were assessed for each condition.

Results: Baseline phenotype distribution was not significantly different between the two genotypes. Higher rate of phenoconversion to IM with duloxetine (71% versus 25%, $p=0.009$) and to PM with paroxetine (94% versus 56%, $p=0.011$) was observed in heterozygous than homozygous EMs. The magnitude of DDI between dextromethorphan and paroxetine was higher in homozygous than in heterozygous subjects (14.6 versus 8.5, $p<0.028$).

Conclusion: Our study suggests that genetic EMs may not represent a homogenous population and that available genetic data should be considered when addressing DDIs in clinical practice.



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BLOOD STEROIDOMICS AS A TOOL TO HIGHLIGHT NOVEL BIOMARKERS FOR THE ATHLETE BIOLOGICAL PASSPORT STEROIDAL MODULE

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The ‘steroidal module’ of the Athlete Biological Passport is currently achieved by GC-MS(/MS) quantification of selected endogenous anabolic androgenic steroids (EAAS) from athletes' urines and represents a key tool for the detection of steroid abuse in sport. Nevertheless, as “urinary steroid profile” suffers from matrix-related limitations such as enzyme polymorphism, bacterial contamination and ethanol consumption, new strategies were recently proposed to improve EAAS doping detection capabilities. Among these, measurement of steroid hormones in blood showed a promising ability to detect testosterone doping and interesting complementarities with the urinary module. A more holistic approach, namely “steroidomics”, could improve the current steroid profile. Therefore, a new “steroidomic” workflow including 5 different steps was developed and will be discussed in the present work:

- 1) SPE-based sample preparation, capable of extracting steroid hormones as well as their phase I and phase II metabolites from serum;
- 2) UHPLC-HRMS method, acquiring Full Scan MS and data dependent MS/MS spectra in both positive and negative ionization modes;
- 3) Data processing including noise filtering, alignment and peak picking followed by normalization;
- 4) Ion annotation with confidence level 1 (identification with authentic standard) and 2 (identification with predicted properties) thanks to an in-house steroid database (350 steroids) together with retention time prediction model;
- 5) Multivariate statistical analysis for highlighting most relevant features;

This workflow was then applied to samples obtained from a testosterone administration clinical trial, with the aim of highlighting new blood biomarkers of both oral and transdermal testosterone intake, similarly to what was previously done on urine matrix.



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Bayesian Genome-Wide Association Study to discover novel lifespan-associated loci

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Identification of genetic variants associated with a phenotype is the first step towards biomarker discovery. However, many Genome-Wide Association Studies (GWASs) are underpowered to detect such variants. Increasing the sample size also increases the GWAS power, but large datasets can be difficult to gather for certain traits, such as lifespan. Leveraging independent sources of information can improve GWAS studies without increasing sample size.

We developed a Bayesian approach to perform an informed GWAS (iGWAS) that accounts for prior information. To estimate effect of single nucleotide variants (SNPs) on lifespan *a priori*, summary statistics of GWASs for related traits, such as education level, smoking or life-shortening diseases have been used. We estimated their causal effect on lifespan using Mendelian Randomization and used it to compute a prior effect for each variant. Our iGWAS, tested on data from 116,000 individuals, identifies novel variants. These variants have been confirmed by a standard GWAS based on >300,000 individuals, demonstrating the reliability of our approach.

So far, our priors have been built using information from summary statistics from other human GWAS, but our iGWAS is adaptable and can be modified to use other types of prior information. As an example, it has been shown that the expression levels of specific genes can be used as a measure of biological age (revealing accelerated aging, hence shorter lifespan) and we are currently working on defining priors based on such gene-expression data.



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Proteogenomic interrogation of human tears using ProQuantum™ High Sensitivity Immunoassay could revolutionize precision medicine in the clinic

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Keywords: *tear biomarker(s), antibodies, precision medicine, ProQuantum™ Immunoassays, ocular diseases, systemic diseases*

Quest for suitable tear biomarkers for diseases has become one of the biggest challenges of today and stands to significantly change clinical practice in future. Human tears with their comprehensive biomolecule repertoire serve as a good source for biomarkers and offer an excellent opportunity for patient stratification in precision medicine. Tear collection is fast, noninvasive and offers a chance to interrogate ocular well as systemic diseases in patients.

Clinicians and translational research scientists studying the multifaceted etiology of various diseases require powerful investigational tools for targeted interrogation of gene activation, cellular interactions, receptor-mediated effects and intracellular signaling processes. Additionally, 'Proteogenomics', or the integration of proteomics with genomics and transcriptomics, is a novel approach that promises to advance translational and clinical research. To this end, ProQuantum™ High Sensitivity Immunoassays incorporating analyte specificity of high affinity antibody-antigen binding with the signal detection and amplification of real-time PCR technologies offers a flexible and scalable qPCR assay platform enabling multidimensional analyses of DNA, RNA, and now biological proteins. Compared to traditional methods, ProQuantum™ Immunoassays offer a greater sensitivity, better dynamic range, smaller sample consumption (1-2 µL per sample), a faster, simplified workflow and robust data analysis software. It is foreseeable that such a technology will have an integral role in medical research and specialized clinics for establishing biomarker profiles of disease status.

It is anticipated that an established 'tear test' would be a very powerful prognostic and / or diagnostic test in eye clinics in the near future for both systemic and ocular disease indication.



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Autologous blood transfusion in sports : emerging biomarkers

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Although it is prohibited by the World Anti-Doping Agency, blood doping through blood transfusion such as autologous blood transfusion, is frequently used by athletes to increase oxygen delivery to muscles and therefore to enhance their performance. Currently, own blood re-infusion is monitored with individual follow-up of hematological variables via the Athlete Biological Passport. With the development of direct quantitative methods, transcriptomics based on microRNA or messenger RNA expression is a promising approach. As blood donation and blood re-infusion alter iron metabolism, quantification of proteins involved in the metal metabolism like hepcidin can be used in an “ironomics” strategy to improve detection of ABT. Alternatively, urine matrix can be used for the quantification of the plasticizer di(2-ethylhexyl) phthalate (DEHP) and its metabolites originating from the bags used to store blood suggesting recent blood transfusion with an important degree of sensitivity and specificity. All these different indirect biomarkers from different sources should be combined using mathematical approaches in a longitudinal monitoring for an improvement of autologous blood transfusion detection. In addition, these biomarkers could increase knowledge and have important implications for the development of prognoses and diagnoses tools in transfusion medicine field.



A metabolomics approach to unravel altered systemic and brain energy metabolism in Alzheimer's disease

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Neurodegenerative disorders, like Alzheimer's disease (AD), are allegedly associated with alterations in central energy metabolic pathways. However, the role of these metabolic alterations, beyond reduced glucose metabolism, remain largely unexplored. We combined untargeted HRMS metabolomics and targeted quantification to unravel metabolic signatures in paired plasma and cerebrospinal fluid (CSF) of AD patients and matched control subjects.

In plasma of AD patients, significantly lower levels of basic, aromatic and branched-chain amino acids were observed as compared to control subjects. The CSF metabolite profiles of AD patients showed lower levels of lysine and higher levels of amino acid catabolites, whilst both plasma and CSF showed higher levels of long-chain acylcarnitines.

The observed differences in plasma and CSF metabolite profiles between AD patients and control subjects strongly suggest perturbed central carbon energy metabolism and induced amino acid catabolism as a possible compensatory response to reduced glucose metabolism in the brain of AD patients.



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The differential plasma proteome of obese and overweight individuals undergoing nutritional weight loss and maintenance

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Background

Profiling of proteomes from human blood plasma via high-throughput quantitative pipelines is key for clinical research and biomarker discovery. Over many years, multiple research groups have pursued the goal of measuring “complete proteomes” with MS (“protein number race”). Much less attention has been given to the “sample number race” that can ultimately lead to sufficient statistical power and deliver robust and reproducible biological findings, taking into account diversity and variability of populations.

Methods

Using a scalable automated workflow to analyze proteins in human samples at clinically-relevant scale and performance, we profiled 1000 clinical samples from the multi-centered dietary intervention study “DiOGenes”, which focuses on preventing and treating obesity through diet.

We generated differential plasma proteome profiles to identify proteins associated with weight-loss and maintenance and explore their relation to BMI, fat-mass, insulin-resistance and sensitivity. Relative protein quantification was obtained before/after weight-loss/maintenance using isobaric tagging MS. T-tests determined differential proteins and linear models explored protein relationships with clinical variables (confounders: center, gender, age).

Results

473 subjects were measured at baseline and 34 weeks; 39 proteins were longitudinally differential. Proteins with higher changes were SHBG, adiponectin, CRP, calprotectin, SAA, and PRG4, whose association with obesity and weight-loss is known. We identified new putative biomarkers for weight-loss and maintenance.

Conclusions

MS-based proteomic analysis of a large cohort of overweight and obese individuals concomitantly identified known and novel proteins associated with weight-loss and maintenance. This is one of the few examples of MS-based proteomics applied to such a large cohort in clinical research.



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Posters abstracts

Evaluation of dried blood spot sampling and UHPLC-HRMS for large-scale screening of drugs and pharmaceuticals

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UMPT³, HUG², UTCF¹

Background: Dried blood spot (DBS) is an attractive solution with respect to invasive classical venepuncture by reducing cost and facilitating storage and shipment. The implementation of high resolution mass spectrometer (HRMS) hyphenated with ultra high performance liquid chromatography (UHPLC) could allow the development of highly sensitive large-scale methods of clinical and toxicological screening using from blood microsampling.

Methods: A UHPLC-HRMS method was developed for DBS analysis on a HRMS instrument (Q Exactive Plus). The samples were analyzed in a full-scan data dependent acquisition (DDA) in positive polarity using an inclusion list currently containing around 1000 substances. Forensic and clinical toxicology cases were then analyzed with this method and cross-validated with a published LC-MS based targeted screening procedures.

Results: Method evaluation was performed on a subset of 32 selected substances covering all classes of interest. Limits of detection (LOD) and identification (LOI) were evaluated. LOD was under 5 ng/ml for almost every substance tested and the extraction process showed good efficiency. Comparison of forensic and clinical toxicology cases analyzed with this method and a published targeted method used for routine analysis was performed. Overall there was a good correlation between the substances detected and identified allowing to cross-validate this method.

Conclusion: The recent development of highly sensitive HRMS allows the use of DBS sampling for large-scale toxicological screening, including the possibility of analyzing THC-COOH and new psychoactive substances (NPS) from few microliters of blood.



UNTARGETED OMICS-BASED DISCOVERY OF EARLY ISCHEMIA INDUCED CHANGES IN Ex Vivo RAT HEART MODEL

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Myocardial ischemia is a complex state characterized by reduced supply of oxygen and nutrients as well as inadequate removal of cellular metabolites. Early myocardial ischemia (EMI) is the time period during the initial hours from the onset of injury that lacks histopathological findings and molecular description defined by a standardized panel of immunohistochemical markers. EMI is a precursor to a variety of ischemic heart diseases and a suspected cause in most sudden cardiac death (SCD) cases. In legal medicine, each year several SCD cases remain unexplained (10% in our center) because these cases lack specific finding. Improving current post-mortem methods to diagnose EMI will allow better assistance of the public prosecutor and the families of the victims. In this study we investigated biomolecular changes in an ex vivo rat model of EMI by performing global untargeted screening with matrix assisted laser desorption/ionization mass spectrometry imaging (MALDI IMS) and multivariate data analysis. Cardiac tissue exposed to ischemia for 15 min, 30 min, 1 h, 2 h, and 4 h was analyzed by MALDI IMS. The results achieved with MALDI IMS were compared to immunohistochemical evaluation of the same samples. The identification of new, specific markers of EMI will not only improve the post mortem diagnosis in cases of SCD but and may also lead to the detection of potential targets for therapy.



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Effect of APOE4 status on cortical thickness co-variance networks in Mild Cognitive Impairment

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Background: Apolipoprotein E epsilon 4 allele (APOE4) is a known genetic risk factor for developing late-onset Alzheimer's disease (AD). In the present study, we assess APOE 4-related effects on the topology of cortical thickness co-variance networks in Mild Cognitive Impairment (MCI).

Methods: We used cortical thickness measures obtained from 253 T1WI images (ADNI database) and graph theory to study brain network properties. The sample comprised 126 APOE4-positive (Carriers) MCI and 127 APOE4-negative (non-Carriers). The differences in regional cortical thickness, global network properties (clustering index, characteristic path length, efficiency, global connectivity), nodal network properties (betweenness centrality), Targeted Attack, and Modularity were compared between groups.

Results: Differences in cortical thickness between groups did not reach statistical significance (FDR-correction). We found in MCI-Carriers compared with non-Carriers lower correlations values between homologous regions as well as global connectivity. The MCI-Carrier showed lower characteristic path length, clustering index and local efficiency but higher global efficiency compared with non-Carriers. MCI-Carriers showed higher network resilience after simulated attacks to regions considered hubs.

Discussion: APOE4 genotype differentially modulates cortical thickness co-variance in MCI. The global connectivity and global efficiency changes in MCI-Carriers may suggest increased presence of long-range connections. On the other hand, lower global network properties and regional centrality in this group suggest a less optimal organization in term of local processing of information. The APOE4 presence was associated with higher network resilience to target attack in MCI. Our findings shed new light on the gene-connectome interaction in AD.



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Combining in vitro neurotoxicity testing with toxicokinetics modelling: a case study of glycol ethers

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Introduction: Glycol ethers (GE) are widely used organic solvents. Among them, Ethylene Glycol Methyl Ether (EGME) is the only one recognized to lead to a reversible central nervous system (CNS) depression in humans, with a tolerance effect typical of organic solvents. For other GEs, symptoms of CNS depression have been observed in animals, but there are not enough data to draw conclusions for humans. Our hypothesis is that other glycol ethers could also be neurotoxic for humans at concentrations often found in the workplace.

Methods: A No-Observed Adverse Effect Concentration (NOAEC) for neuronal toxicity was derived from in vitro data obtained using a 3D rat brain model for three GEs: propylene-glycol methyl ether (PGME), propylene glycol butyl ether (PGBE), and EGME as positive control. A physiologically-based toxicokinetic (PBTK) model was developed to predict an air concentration that could lead to a brain concentration greater than the NOAEC.

Results and conclusions: Among the three GEs tested PGBE had the lowest NOAEC, thus it is potentially toxic for human brains; PGME was likely not to be neurotoxic at the current occupational exposure level (OEL); and finally, EGME was not neurotoxic at any of the tested concentrations. This suggests that its main metabolite, methoxyacetic acid, which causes toxic effects for development and reproduction, could also be responsible for EGME's known brain toxicity.



Three times a week and five times a week walking exercise training is equally effective in improving walking capacity in a mouse model of peripheral artery disease

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Peripheral artery disease (PAD) is characterized by the narrowing of arteries supplying the lower limb secondary to atherosclerosis. This leads to a decreased blood flow, and eventually to ischemic-muscle cramping-pain during exercise, resulting in walking capacity (WC) impairment. Supervised walking exercise (SWE) at least 3x/week is the first line therapy to improve WC in PAD patients. However, the optimal SWE frequency maximally improving WC remains unknown.

Aim: Compare three versus five SWE sessions/week in improving WC in a mouse model of PAD.

Design/Methods: Hindlimb ischemia generated in C57BL/6 apolipoproteinE^{-/-} atherosclerotic mice by right common iliac artery ligation. After surgery (baseline), mice were divided into three groups: sedentary control (SED), SWE 3x/week (SWE3x), and SWE 5x/week (SWE5x).

SWE: treadmill walking for 7 weeks (40min/session, 20cm/sec).

WC: maximal walking distance (MWD, meters) using a treadmill running incremental test to exhaustion.

Ischemic limb perfusion: laser Doppler imaging.

Real-time RT-PCR: on ischemic quadriceps (QR) muscles to assess muscular inflammation profile.

Results: MWD significantly improved in SWE3x (baseline: 311±67m vs. end of the study: 492±170m) and SWE5x mice (267±100m vs. 491±304m); the improvement extent did not significantly differ between the groups (SWE3x: 58% vs. SWE5x: 83%). mRNA expression of pro-inflammatory cytokine IL-1B in QR significantly decreased by 53% in SWE3x compared to SED. CD11c/CD206 mRNA expression ratio (pro-inflammatory M1/anti-inflammatory M2 macrophage balance marker) significantly decreased in SWE3x and SWE5x, in comparison with SED.

Conclusion: 3x/w and 5x/w SWE are equally effective in improving WC in a mouse model of PAD. SWE modulates muscular inflammatory status.



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MitoRS, a method for high throughput, sensitive, and accurate detection of mitochondrial DNA variants

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Mitochondrial dysfunction has been linked with many pathologies. In several instances, this could be associated with polymorphism in one of the > 1'000 nuclear DNA encoded genes involved mitochondrial function. However, it is still technologically challenging to perform large scale analysis of the mitochondrial DNA (mtDNA) itself, and the contribution of mtDNA encoded variants is still poorly understood.

We have developed MitoRS, a method tackling these challenges by applying next generation sequencing for universal, high throughput, robust, sensitive and accurate detection of mtDNA variants. Fine-tuned parameters are applied in the analysis to allow detection of variants even of low frequency heteroplasmy. We anticipate this method will promote the systematic analysis of mtDNA polymorphisms and may help assessing the impact of mtDNA heteroplasmy on metabolic health, brain function, cancer progression, or ageing.



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Use of global metabolomics for the identification of new CYP2D6 endogenous biomarkers

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CYP2D6 metabolizes ~25% of all marketed drugs. There is an important variability in the activity of this enzyme among individuals. The cause of this variability might be environmental, genetic, ethnical or even related to a disease. The administration of a CYP2D6 probe drug (e.g. dextromethorphan) is a good way to characterize CYP2D6 phenotype. Nonetheless, exogenous probes can induce adverse effects despite using sub-therapeutic microdoses. In addition, the vulnerable population (e.g. pregnant women, neonates, children) cannot be phenotyped in this manner. Therefore, finding an endogenous substance which is metabolized by CYP2D6 could replace usual phenotyping procedure using a probe drug. This study uses global metabolomics to find an endogenous metabolite of CYP2D6.

Plasma collected from 33 healthy volunteers before and after administration of paroxetine, a strong inhibitor of CYP2D6, are analysed by metabolomics using a LC-Q-Exactive HRMS. After data normalization, multivariate analysis was performed to select potential endogenous metabolites which are metabolized by CYP2D6. Biomarkers with VIP scores higher than 1 and fold-change of normalized intensities in control phase versus inhibition > 1.5 are selected.

In ESI+ and ESI- modes, 4 and 18 features, respectively, can be identified on the basis of the criteria previously described and might correspond to potential endogenous biomarkers of CYP2D6.

Complementary strategies will be used to characterize the features identified in this study, including the use of databases (e.g. METLIN Metabolite Database), MS/MS fragmentation patterns, NMR and comparison with a reference standard. Confirmation with in-vitro tests (e.g. supersomes) will be performed to clarify the metabolic pathway.



Nrf2 couples antioxidant defense with thyroglobulin production and iodination in the thyroid gland.

Sykiotis G.

CHUV

The thyroid gland has a special relationship with oxidative stress. While generation of oxidative substances is part of normal iodine metabolism during thyroid hormone synthesis, the gland must also defend itself against excessive oxidation in order to maintain normal functionality. Although a number of antioxidant and detoxification enzymes presumably aid thyroid cells to maintain homeostasis by ameliorating oxidative insults, including under conditions of exposure to excess iodine, the factors that coordinate their expression with the cellular redox status are not well known. Across the evolutionary spectrum, the ubiquitously expressed transcription factor Nrf2 is required to defend tissues against oxidative stress thus protecting organisms against pathologies that relate to DNA, protein and/or lipid oxidative damage. Using ubiquitous and thyroid-specific Nrf2 knockout (Nrf2-KO) mice and Nrf2- or Keap1-KO thyroid follicular cell lines, we show here that Nrf2 mediates antioxidant transcriptional responses in thyroid cells and protects the thyroid from oxidation induced by iodine overload. Surprisingly, we also found that Nrf2 has a dramatic impact on both the basal and the thyrotropin-inducible intra-thyroidal abundance of thyroglobulin (Tg), the precursor protein of thyroid hormones. This effect is mediated by cell-autonomous regulation of Tg gene expression by Nrf2 via its direct binding to two evolutionarily conserved antioxidant response elements in an upstream enhancer. Yet, despite upregulating Tg levels, Nrf2 limits Tg iodination both under basal conditions and in response to excess iodine. Nrf2 thus couples cell stress defense mechanisms to iodine metabolism and the thyroid hormone synthesis machinery.



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Tears: a future vision for the biomarker discovery in medicine?

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Background:

Tears represent an exciting promising fluid for biomarkers discovery. Indeed, their easy way to collect, invasiveness, timeless and absence of side effects make them particularly attractive for clinical uses. Their complex molecular composition is highly regulated depending on several factors including ocular and systemic diseases. Here we present an in-depth analysis of the healthy human tear composition, but also some clinical applications including both ocular and systemic diseases, reflecting the interest of this fluid for biomarker discovery.

Methods:

Tears of healthy subjects and patients were collected with Schirmer's papers. Trypsin digestion and off-gel electrophoresis fractionation were performed then analyses were done using high throughput mass spectrometers coupled to a liquid chromatography. In the context of disease studies, quantification experiments were performed to compare the protein levels between patients and controls. Bioinformatics software were used to analyse the data.

Results:

Globally 2021 proteins were identified in human tears with 2 unique peptides. Pathway analyses highlighted the glycolysis and the coagulation and complement cascades. Performing quantification analyses, the glycolysis was found as deregulated in patients suffering from some ocular diseases such as thyroid-associated orbitopathy (TAO) or central serous chorioretinopathy. Moreover, some proteins related to the inflammation were strongly identified as potential biomarkers for the multiple sclerosis or TAO.

Conclusion

This in-depth analysis of tear proteome drastically improved the knowledges of this fluid. Furthermore, thanks to their ability to reflect biological deregulations related to ocular and systemic disease contexts, tears clearly confirm their place among the promising future diagnostic strategies.



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Genome-wide association study (GWAS) of central pain sensitization in fibromyalgia patients

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Fibromyalgia syndrome (FMS) covers a spectrum of chronic pain conditions characterized by widespread pain and increased sensitivity to nociceptive stimulus or tenderness. Central sensitization is thought to be one of the key mechanisms underlying FMS. This process can be described as a loss of the natural balance between transmission of pain stimuli to the CNS and the central pain inhibitory feedback mechanisms. While familial aggregation could suggest a potential genetic component in FMS development, isolation of genetic determinants has proven difficult due to the multi-factorial nature and complexity of the syndrome.

In this project, we will use two decades clinical and genetic material to investigate more than 550'000 human genetic polymorphisms in 302 FMS patients and healthy controls. The bioinformatics analysis will combine genome-wide and candidate gene approaches. Further refinement in the analysis will be provided by matching case-controls, interaction between covariates and population stratification. Our primary stratification determinant for central pain will be the nociceptive flexion reflex (NFR) or RIII threshold which is significantly lowered in FMS patients.

Thus, the genome-wide characterization of our cohort will be used to confirm suspected genetic predisposition and identify new genetic determinants of FMS based on objective measurements of central sensitization. Follow-up work combining genomic, transcriptomic and proteomic techniques will aim to gain a better understanding of the various pathophysiological mechanisms underlying the disease and allow the development of more optimized and better targeted personalized treatments for FMS patients.



Proposed study-design for efficacy of nicotine delivery from electronic cigarettes (ECs)

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Introduction: New nicotine delivery systems have emerged, claiming lower risks compared to conventional cigarettes (CC), such as the electronic cigarette (EC). These are popular amongst the general smoking population, and especially to those wishing to quit smoking. Few studies have measured the efficiency of nicotine delivery to the EC user. This pilot study aims to evaluate the rate of nicotine delivery in blood from vaping ECs that will subsequently help in assessing their efficiency as aid towards smoking cessation.

Method: We will recruit dual users (CCs and ECs) and EC-only users. Dual users will use a CC and an EC of their choice from the two devices selected for this study (Nautilus and GS-Air). EC users will use both ECs and chose from two different nicotine concentrations. Volunteers will smoke/vape in a controlled environment (exposure chamber at IST) and blood will be collected at the following time-points mentioned bellow to monitor the kinetics of nicotine:

- CC: baseline, 0, 1, 3, end of use, and at minutes 8, 16, 30 after end of use
- EC: baseline, 0, 5, 10, 20, 30(end of use) and at minutes 10, 30, 40 after end of use

At the end of the use, the volunteers will fill in a QSU-brief questionnaire to evaluate their urge to smoke again and questionnaires stating the satisfaction from using the device.

Expected outcome: Combining the nicotine blood levels with the results of the questionnaires will give us an overall picture on how ECs perform in comparison to conventional cigarettes in regards to maximum nicotine concentration, peak and elimination time. This study will stand as a stepping stone for an upcoming study to determine if and how ECs can successfully be part of cessation method procedures.



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BrainLaus - MRI imaging in the longitudinal population-based cohort CoLaus

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Objectives: Investigation of endogenous and environmental factors that determine cognitive and motor performance in healthy ageing while monitoring in vivo brain microstructure. The main goal is to create accurate and reliable predictors for healthy ageing and neurodegeneration.

Materials and methods: CoLaus and its extension PsyCoLaus represent a longitudinal cohort of 6,188 individuals recruited from the Lausanne population that started in 2003. Participants were deep phenotyped to create an overwhelming amount of somatic, neurocognitive and psychiatric data. On the second follow-up of PsyCoLaus we initiated the neuroimaging sub-project BrainLaus focused on brain anatomy imaging with > 1100 participants. For brain anatomy assessment, we use unique quantitative magnetic resonance imaging (MRI) protocols indicative for brain tissue properties – iron, myelin and tissue water beyond the established metrics of cortical thickness and volume. Here, we present the main characteristic features of the obtained brain MRI data and give examples of descriptive analysis on lifespan trajectories and cognitive performance.

Results: The MRI cohort (n=1040) consists of 51% female and 49% male individuals at age 49.6±9.1 at baseline and 60.3±9.5 on MRI follow-up. We observe differential distribution of educational level, job activity, alcohol consumptions and metabolic changes between male and female participants. In a smaller subsample with longitudinal neurocognitive data (n=71) with elderly participants (age 68.4±2.9 at baseline and 74.0±2.9 at scanning) we show performance decline related brain anatomy differences.

Conclusion: The BrainLaus sample of the PsyColaues cohort provides a unique opportunity to investigate brain anatomy changes along many axes of research. The innovative imaging protocol offers the possibility for deep phenotyping of ageing related changes in a way that allows for a straightforward neuro-biological interpretation.



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Analytical Coverage Investigation of a Database of Reference Compounds for Metabolomic Studies

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Metabolomic sciences aim at the study of low molecular weight molecules in different biological matrices. Although the investigation of the whole metabolic content can be achieved by means of untargeted approaches, the annotation of relevant metabolites is necessary for an optimal interpretation of the biological information present in the studied systems^[1]. In order to make possible the identification of features, a chemical library containing above 630 standard metabolites (Mass Spectrometry Metabolite Library of Standards from Sigma-Aldrich), was investigated in our laboratory with generic HILIC and RPLC methods hyphenated to MS in positive and negative acquisition modes.

LC methods were developed on a Waters H-Class Acquity system, using Waters Acquity UPLC® BEH Amide (HILIC) and Phenomenex® Kinetex C18 (RPLC) stationary phases. MS analysis was conducted on a Bruker maXis 3G QTOF. Both methods were chosen owing to their orthogonal retention properties^[2]. The quality of the chemical information delivered by each method was carefully evaluated, as well as the overlap among them based on Derringer's desirability functions, established on different criteria such as retention, sensitivity and peak shape.

Computed scores allowed to spot the best method(s) for the analysis of every detected compound. Furthermore, the coverage and complementarity of the methods was further evaluated. As a result, the combination of all LC methods and ionization modes led to an overall coverage of 80% of the proposed library. It was found that when only two or three out of the four analytical methods were carefully chosen, the obtained coverage decreased by only 10%.

Finally, the most informative set of LC methods and ionization modes was applied to the investigation of biological matrices, and the annotation of metabolites was carried out by using the constructed database.

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Toward a better understanding of chronic kidney disease using metabolomics

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Background: Metabolomics aims to analyse comprehensively the metabolic complexity of biological systems and constitutes a potent method for assessing phenotype modifications caused environmental influences or pathologies. An extensive coverage of metabolites (mass < 1000 Da) is required for relevant untargeted metabolomics. As no technique offers an exhaustive monitoring of all metabolites in a biofluid, the use of multiple analytical platforms is needed. This methodology was implemented in the context of a clinical study to detect alterations in plasma metabolomic profiles due to chronic kidney disease (CKD) and allow a better understanding of the pathology.

Methods: Reversed-phase chromatography (RPLC) and hydrophilic interaction chromatography (HILIC) coupled to high resolution mass spectrometry (HRMS) are complementary techniques commonly used for their coverage of apolar and polar metabolites, respectively. A strategy based on the combination of these two analytical approaches was applied to plasma samples collected from a clinical study designed to evaluate the metabolic impact of CKD. The cohort was composed of 56 control samples and 104 patients at several disease stages. Each sample and quality control (QC) was analysed by RPLC and HILIC coupled to QTOF-MS in negative and positive ESI mode.

Results: More than 300 annotated compounds were investigated thanks to the fusion of datasets generated from multiple platforms using an in-house database of 600 metabolites. The major sources of variability observed in the dataset were related to biological alterations due to the pathology that could be related to the Glomerular Filtration Rate (GFR). The multivariate analysis of the dataset showed a strong ability to stratify patients.

Conclusions: The workflow developed in this study allowed patient stratification according to CKD stages and helped to generate biological hypotheses based on the metabolomic profiles.