7th EGID SYMPOSIUM

27-28th November, 2023
Lille - FRANCE

Programme & Abstract book

Content

November 27th - 28th, 2023 - Lille Grand Palais

Egid
European Genomic Institute for Diabetes

7th EGID SYMPOSIUM

E.G.I.D.
Best Poster Award
1000 €
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October 26th, 2023

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7th EGID SYMPOSIUM

NOVEMBER 27th 2023

14.00 **Registrations**

14.20 **Welcoming & Introduction: Prs Philippe FROGUEL, François PATTOU, Bart STAELS**
A Look Back at 15 Years in the Service of Research and Patients
And Tomorrow? What Ambitions for Research, Patients, and the Region?

16.00 Coffee Break

16.30 Special Guest: Pr Francisco RUBINO, London, UK
Is Obesity a Disease? If so, How Can we Diagnose It?

17.30 **Posters session and Cheese & Wine**

NOVEMBER 28th 2023

09.00 **Welcoming**

09.10 Keynote speaker: Pr Johan AUWERX, Lausanne, SWISSZERLAND
Cross-Species Genetics to Map New Players in Mitochondria and Aging

Session 1: Adipose Tissue

09.50 Pr Susanne MANDRUP, Odense, DENMARK
Enhancer Networks Regulating the Adipogenic Master Switch

10.30 Pr Camilla SCHEELE, Copenhagen, DENMARK
Brown Fat in Human Metabolism

11.10 Coffee Break

11.40 Pr Philipp E. SCHERER, Dallas, USA
The Unsurpassed Potential of Adipose Tissue to Influence Metabolism

12.20 Lunch

Session 2: Innovative Treatments

13.30 Pr Matthias TSCHÖP, Munich, GERMANY
Overcoming Obesity: The Discovery of Multi Receptor Drugs

14.10 Pr Zachary GERHART-HINES, Copenhagen, DENMARK
Dual Energy Expenditure and Appetite Control by Insulin-Sensitizing NK2R Agonism to Treat Cardiometabolic Disease

14.50 Pr Karine CLEMENT, Paris, FRANCE
Toward Precision Medicine Approach in Severe Obesity

15.30 Coffee Break

Session 3: Animal and Human Genetics

16.00 Pr Antje KÖRNER, Leipzig, GERMANY
A New Human Obesity Trait Mimicking the Agouti Mouse Model of Obesity

16.40 Pr Melina CLAUSSNITZER, Boston, USA
Converting Metabolic Risk Variants-to-Function (V2F) Using Adipocytes as a Model System

17.20 **Best Poster Award and Conclusions**

Speakers Abstracts

Johan Auwerx,
MD. Ph.D.
Ecole Polytechnique Fédérale de Lausanne – Swiss Federal Institute of Technology in Lausanne
Lausanne, SWISSZERLAND

> Cross-species genetics to map new players in mitochondria and aging

Our understanding of genetic mechanisms that define complex traits has been hindered by the difficulty of obtaining comprehensive omics datasets across a broad range of biological “layers”. Complete data on the genome of individuals can be readily obtained, but the full complexity of the transcriptome, proteome, metabolome, and phenome have remained largely out of reach in humans. This is, however, beginning to change, with the development of robust multi-layered omics strategies that are pioneered in model organisms. I will here show data on the characterization of healthspan—in the BXD mouse genetic reference population, in 8 collaborative cross (CC) founder strains, and in a large F2 cross of 4 CC founders— and of lifespan in a population of UM-HET3 mice of the NIA’s Intervention Testing Program (ITP). Large variability was observed across all omics layers in these studies. We exploited multilayered sets of molecular phenotypes—genomics, transcriptomics, proteomics, and metabolomics—together with clinical phenotypes to identify candidate genes and pathways that define mitochondrial function and health- and lifespan traits. Data from mouse population studies can be seamlessly integrated with observational human genetic studies, such as available through the UK and Estonian biobanks. With such a cross-species multi-omics strategy, causal variants were identified in proteins (e.g.,Mrps5, Dhtkd1, Cox7a2l, Ubp1) and protein networks (e.g., NAD+ homeostasis, mitochondrial stress pathways, inflammation) involved in the control of health- and lifespan. Our large-scope multi-omics measurements in mouse populations combined with cross-species validation hence provided us with robust conserved and mechanistically defined pathways that underpin complex traits involved in health and aging; such protein networks provide actionable targets for anti-aging interventions.

Melina CALUSSNITZER

PhD, Assistant Professor of Medicine, Harvard Medical School
Investigator, Center for Genomic Medicine and Endocrine Division Massachusetts General Hospital
Institute Member, The Broad Institute of MIT and Harvard
Co-Director, Type 2 Diabetes Systems Genomics Initiative, Broad Institute of MIT and Harvard
Associate Director, Scientific Strategy, Novo Nordisk Foundation Center for Genomic Mechanisms of Disease at the Broad Institute
Boston, USA

> Converting Metabolic Risk Variants-to-Function (V2F) using Adipocytes as a Model System

The Claussnitzer team is enthusiastic about adding function to large-scale genetic association study results (Variant-to-Function, V2F) in the context of metabolic disease. The motivation of our research program has been that genetic studies succeeded in identifying 1,000s of associations between genetic loci and metabolic disease in humans. Yet, the next grand challenge — systematically dissecting the mechanisms by which these variants affect disease — has still to be solved and scaled. We have previously developed V2F frameworks for going from variants to genes to cells to biological pathways for the FTO obesity risk locus, and shown that this framework generalizes to other genetic risk loci. In this presentation, I will introduce novel experimental and computational strategies to map cellular processes in the context of type 2 diabetes genetic variation.

Karine CLÉMENT

Prof, *INSERM/Sorbonne University "NutriOmics" Research Unit and Department of Nutrition, APHP, Pitié-Salpêtrière hospital, Paris.*
Paris, FRANCE

> Toward precision medicine approach in severe obesity

The genetics of obesity is a complex field that has progressed enormously in recent years. Monogenic obesity is caused by a mutation in a single gene and is inherited within a family or sometimes occurs de novo. This type of obesity is relatively rare, even ultra-rare, but the phenotype is severe, beginning in early childhood, very often associated with other symptoms, such as severe eating disorders and/or neurodevelopmental disorders. Multidisciplinary approaches are therefore essential in these pathologies.

Considerable progress has been made in the diagnosis and treatment of rare obesity by acting on the hypothalamic leptin-melanocortin pathway.

Obesity linked to bi-allelic variants, upstream of the MC4R, in the leptin/melanocortin pathway, are indeed indications recognized by the American and European regulatory agencies for this treatment. France benefits from early access to this treatment for patients with pathogenic variants of LEPR, PCSK1 and POMC, and more recently Bardet Biedl syndrome. The notable effect of setmelanotide is a weight loss of more than 10% of initial weight in most cases (mean loss of 25.6% for POMC variants and 12.5% for LEPR variants), with a reduction in hunger scores of 27.1% and 43.7%, respectively. In patients with BBS, approximately 30% responded to treatment with approximately 10% weight loss at one year and 60% with 5% weight loss at one year. An improvement in quality of life was also observed in these patients with setmelanotide. In the future, more patients with genetic variants in the melanocortin pathway could be candidates for these treatments if the results of the ongoing clinical trials are successful. We are therefore at the heart of the challenges of precision medicine in this field.

Zachary GERHART-HINES
(Ph.D. Biochemistry-Cellular and Molecular Biology) est Associated Professor, University of Copenhagen
Copenhagen, DENMARK

> Dual energy expenditure and appetite control by insulin-sensitizing NK2R agonism to treat cardiometabolic disease

Simultaneously reducing food intake and boosting energy expenditure represents a powerful strategy for counteracting cardiometabolic diseases, such as obesity and type 2 diabetes. However, current approaches require combining different receptor agonists to both decrease energy intake and promote oxidative metabolism. Here we show that activation of the Neurokinin 2 Receptor (NK2R) is sufficient to dually suppress appetite via the central nervous system and increase energy expenditure and insulin sensitivity in the periphery. We find that human genetic variants in NK2R are significantly linked to indicators of cardiometabolic health. Yet assessing the therapeutic potential of NK2R signaling has long been hindered because its endogenous ligand, Neurokinin A (NKA), lacks receptor specificity and has a markedly short half-life. Therefore, we engineered highly selective, long-acting NK2R agonists with the capacity for up to once-weekly administration in humans. NK2R activation induces energy expenditure and reduces food intake and body weight in both diet-induced and genetic rodent models of obesity. Moreover, hyperinsulinemic-euglycemic clamp studies show that NK2R agonists act as potent insulin sensitizers. In diabetic, obese nonhuman primates, NK2R agonism decreases food intake, body weight, triglycerides, glucose, and cholesterol, and corrects insulin resistance, without nausea or adverse cardiovascular effects. These results define a conserved role of NK2R agonism to improve systemic energy homeostasis and reveal a single receptor that can both leverage energy-expending and appetite-suppressing programs to counteract cardiometabolic dysfunction.

Antje KÖRNER

Prof, *Center of Pediatric Research, University Childrens Hospital Leipzig, University of Leipzig*

Helmholtz Institute of Metabolic, Adiposity and Vascular Research (HI-MAG) Leipzig, Helmholtz Munich.

Leipzig, GERMANY

> A new human obesity trait mimicking the Agouti mouse model of obesity

Multiple factors are responsible for the development and progession of obesity. Genetic factors play a role, although this is still poorly understood. Besides the acknowledged polygenic background of “common” obesity, it is important to identify the rare cases of monogenic obesity.

We identified a novel cause of monogenic severe childhood obesity. A genetic rearrangement at the ASIP (agouti-signaling protein) gene locus causes aberrant ectopic ASIP expression in a patient with extreme childhood obesity. The patient’s clinical presentation with early-onset obesity, overgrowth, red hair, and hyperinsulinemia is concordant with that of so called “agouti mice” ubiquitously expressing the murine ASIP-homolog nonagouti. Functionally, ASIP represses melanocortin receptor activation that regulates eating behavior, energy expenditure, adipogenesis, and pigmentation. As such, ASIP dysregulation can cause obesity. Importantly, this offers the opportunity to treat these patients with MC4R agonists. The type of mutation escapes standard genetic screening algorithms and many patients would be undetected. With targeted screening, we already identified 8 additional patients with the identical mutation from the Leipzig childhood obesity cohort. This implies that ASIP mutations may constitute the most frequent of the rare monogenic obesity traits after mutations in the MC4R gene itself. We have established a „humanized mouse model“ carrying the human ITCH-ASIP chromosomal rearrangment. These mice show a phenotype of obesity, hyperglycemia and fatty liver.

Taken together, ubiquitous ectopic ASIP expression is a novel, frequent and potentially treatable monogenic cause of human obesity that so far escaped detection. This does not only help to better understand genetic causes of obesity; it shows the necessity to change conventional genetic screening approaches to facilitate diagnosis of these patients and offer them treatment opportunities.

Susanne MANDRUP
Center for Functional Genomics and Tissue Plasticity
Functional Genomics & Metabolism Research Unit, Dept. Biochemistry & Molecular Biology, University of Southern Denmark
Odense, DENMARK

> Enhancer networks regulating the adipogenic master switch

Adipocytes develop from fibroblastic progenitor cells in the perivascular niche. The transcriptional network driving this differentiation has been extensively studied in vitro and has been shown to involve a first wave of transcription factors that induce major chromatin remodeling as well as the expression of the second wave of adipogenic transcription factors, including the master regulator peroxisome proliferator-activated receptor γ (PPARγ). Using enhancer capture Hi-C combined with genome-wide mapping of transcription factors and enhancer activity, we showed that dynamic enhancer-enhancer interactions appear to play an important role in lineage specific gene activation. Enhancer communities, i.e., groups of enhancers that interact more often with each other than with other regions in the genome, containing highly connected enhancers (HICE communities) appear to act as hubs driving lineage-specific gene expression.

The *PPARG* locus is associated with a HICE community which is preestablished but inactive in the stem cell state. Upon induction of differentiation, additional enhancers are activated and become part of the HICE community. Systematic deletion of individual putative enhancers in this locus shows that many enhancers play an important and non-redundant role in activation of *PPARG* expression during differentiation. Our results demonstrate elaborate crosstalk between enhancers driving expression of this important lineage-determining gene. Genetic variants associated with human metabolic diseases map to these enhancers.

Francesco RUBINO

MD, Professor of Metabolic and Bariatric Surgery, King's College London
Chair, Lancet Commission on Clinical Obesity
London, UK

> Is Obesity a Disease? If so, How Can we Diagnose It?

The notion that obesity is not merely a risk factor for various illnesses, such as diabetes, but a disease in its own right, has gradually been recognized by several medical societies and other organizations, including WHO, the European Commission among others.

The idea of obesity as a stand-alone disease entity, however, remains highly controversial, both within and beyond medical circles.

Those who oppose the idea of obesity as a disease point to the fact that body mass index (BMI) is not a clinical parameter but a surrogate (and imprecise) measure of adiposity-related risk of future disease or increased mortality. They argue, legitimately, that a risk factor is not a disease and that measuring obesity merely by BMI, as we do today, can both under- and over-diagnose disease. In this context, a blanket definition of obesity as a disease would render a large proportion of the population, including people with relatively preserved health, immediately eligible for expensive treatments or claims of disability, effectively making obesity a socially intractable problem in many countries.

On the other hand, the lack of recognition of obesity as a disease means that many patients with objective ill-health continue to face significant barriers to access to potentially life-saving therapies.

Developing an objective definition of disease in obesity and reliable criteria for its diagnosis would help facilitate a more rational and systematic approach to the problem and improve identification of appropriate targets for prevention vs. treatment strategies. Such re-framing of obesity could also help overcome misconceptions that reinforce weight-based bias and stigma.

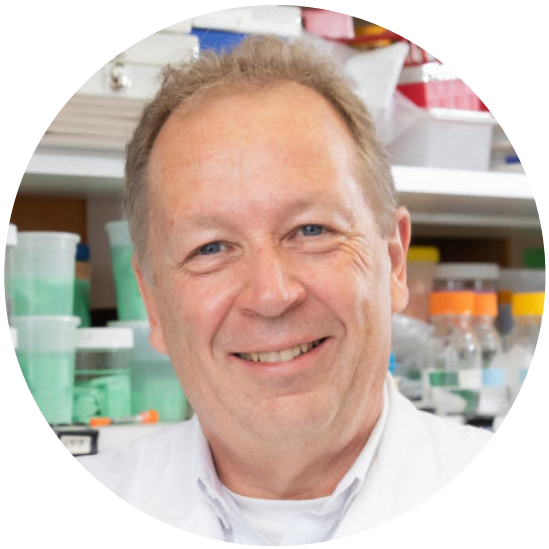
To tackle this issue, the Lancet Commission on Clinical Obesity has been tasked with the development of a clinically-meaningful definition of disease in obesity and the identification of evidence-based criteria for its diagnosis.

This presentation describes the rationale for the Lancet Commission on Clinical Obesity and the complexity of addressing this issue.

Camilla SCHEELE
MSc, PhD - University of Copenhagen, Associate Professor
Novo Nordisk Foundation Center for Basic Metabolic Research
Copenhagen, DENMARK

> Brown fat in human metabolism

Body fat distribution and adipocyte functionality are determinants of metabolic health in a depot-dependent fashion. The ability to activate brown fat (BAT) in response to cold, associates with cardiometabolic health, whereas accumulation of lipids in the visceral adipose depot associates with cardiometabolic disease. The underlying mechanism for these correlations remains elusive and motivates further comparisons of adipose depots in humans. If BAT plays an active role in metabolic regulation, restoring this function could improve human metabolic health. We are investigating metabolic regulators secreted from adipose tissue, in a depot dependent manner. With recent insights in the heterogeneity of adipose tissue, it is important to study this at a single cell resolution. In this respect, we found that progenitor cells from four human brown and white adipose depots, separate into two main cell fates during early differentiation, adipogenic cells and so-called SWAT cells. We are currently studying how these two cell fates diverge in white and brown adipose depots in humans and how this might be related to metabolic disease.



Philipp E. SCHERER
Prof, *Touchstone Diabetes Center, University of Texas Southwestern Medical Center.*
Dallas, USA

> The Unsurpassed Potential of Adipose Tissue to Influence Metabolism

Obesity is the condition characterized by excessive fat accumulation in adipose tissue and is a risk factor contributing to overall mortality, by increasing the incidence of T2D, dyslipidemia, cardiometabolic disease, cancer and morbidity and mortality, due to infectious diseases, including Covid19. The adipocyte takes center stage in the dysregulation of adipose tissue. Through manipulation of specific signaling pathways (e.g. the GIP Receptor axis) or direct manipulation of critical secretory factors (adiponectin, leptin and endotrophin), we are targeting the adipocyte from multiple different angles. The adipocyte exerts a metabolically dynamic role: it adapts and expands to store for excess energy and serves as an endocrine organ capable of synthesizing a number of biologically active molecules that regulate metabolic homeostasis. Chronic obesity upends a delicate balance of extracellular matrix synthesis and degradation, and the ECM accumulates such that it prevents the plasticity and function of the diverse cell types in adipose tissue. This starts a series of maladaptive responses among the cells in adipose tissue which leads to inflammation and fibrosis, major mechanisms that explain the link between obesity and insulin resistance, risk of type 2 diabetes, cardiovascular disease, and non-alcoholic fatty liver disease. Adiponectin, leptin and endotrophin lend themselves for targeted manipulation towards upregulation (adiponectin) or downregulation (leptin, endotrophin) the results of which have important systemic consequences. Implications of these systemic manipulations will be discussed.

Matthias TSHÖP

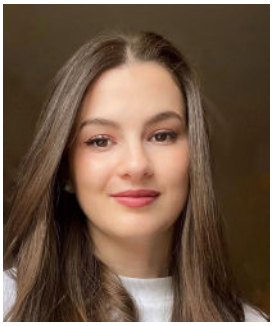
Prof, *CEO and Spokesperson for the Management at Helmholtz Munich*

Réponse mail: CEO and scientific director at Helmholtz Munich

Munich, GERMANY

> Overcoming Obesity: The Discovery of Multi Receptor Drugs

Metabolic disease research suggests that chemistry based on more than one endocrine factor may be required to significantly reduce body fat in patients with severe metabolic syndrome. Combinations of specific gut hormones appear to offer the level of potency required for development of pharmacology based on this principle, as indicated for example by insights emerging from bariatric surgery research. Over the last 20 years, we have tested a large number of combination therapies based on multiple gastrointestinal and adipocyte derived signals. Balanced single molecule peptide hormone based GIP-GLP1 as well as glucagon-GLP-1 co-agonists caused loss of body fat and glucose metabolism benefits in mouse models of obesity and diabetes in a way that was superior to any established mono-agonists. Translational data now confirm and expand best-in-class efficacy ofGIP-GLP1 co-agonists in clinical trials and the first GIP-GLP1 coagonists have recently been submitted for FDA approval. Since co-infusion of a soluble and stable glucagon mono-agonist in parallel with GIP-GLP1 co-agonist treatment provided additional benefits, we went on to discover, test and develop single molecule GIP-GLP1-glucagon triple-co-agonists. These novel tri-agonists again showed unprecedented metabolic and body weight benefits in mouse and rat models of obesity and diabetes. In pre-clinical trials, triagonists were significantly moreefficacious than GIP-GLP1 and early clinical studies now show these types of triagonists to be safe and potent. Almost a decade after Matthias Tschöp reported his discovery of a new drug class of GIP/GLP-1 dual agonists (Science Translational Medicine 2013), the first such dual GIP/GLP-1 agonist - Eli Lilly's "Mounjaro" (tirzepatide) - was FDA-approved for the treatment of type 2 diabetes and is under review for approval for obesity. Several other versions of these dual and triple agonist classes of drugs are successfully underway through clinical phase I-III trials



Mouna ACCARY

Study of the effects of jet lag on brown adipose tissue

Mouna Accary[1], Aurore Hebras[1], Christian Duhem[1], Mélissa Leriche[1], Stéphane Delhaye[1], Yasmine Sebti1, Benoit Pourcet[1], Bart Staels[1], Hélène Duez[1], Alicia Mayeuf-Louchart[1]

[1]*Univ. Lille, Inserm, CHU Lille, Institut Pasteur de Lille, U1011- EGID, F-59000 Lille, France*

Scientific context : Obesity is a global health issue which is linked to other metabolic diseases. The discovery of active brown adipose tissue (BAT) in adult humans has renewed interest in its targeting to treat metabolic diseases. BAT activity is inversely correlated with body mass index and is reduced in obese patients. BAT shows circadian activity, leading to 24-h body temperature variations. Circadian rhythm disruption affects metabolism and cardiovascular health. However, the impact of biological clock alterations on BAT is less understood.

Objective : This study investigates the jet lag (JL) effects on BAT in mice.

Materials & methods : Mice underwent a 12 h JL with phase inversion every week for 14 weeks and were sacrificed every 3 hours for 24 hours or at a single point after 14, 18 and 34 weeks of JL. Gene expression, morphology and BAT activity were analyzed.

Results & discussion : Our results show rhythmic changes in lipid droplets size over 24 h in control mice. This rhythm is altered after jet lag, resulting in an increase in lipid droplets size at all times of the circadian cycle. The impact of jet lag on lipid droplets intensifies over time, gradually increasing lipid droplets size during the different JL durations. Moreover, JL alters mRNA expression of genes involved in lipid metabolism and inflammation. Finally, it reduces the uncoupled respiration of BAT, indicating impaired BAT activity in these conditions.

Conclusion & perspectives : All our results suggest that JL induces BAT whitening. More analysis at morphological and transcriptional levels will allow to better understand the underlying mechanisms.

Cécilia BELLENGIER

The nuclear receptor Rev-erb-α controls angiogenesis and intraplaque neovascularization in atherosclerosis

Bellengier C[1], Ferri L[1], Julla JB[3], Bongiovanni A[2], Delhaye S[1], Duhem C[1], Thorel Q[1], Hebras A[1], Ram B[1], Mayeuf A[1], Sebti Y[1], Venteclef N[3], Tardivel M[2], Staels B[1], Cantelmo AR[1], Gautier JF[3], Duez H[1] and Pourcet B[1].

[1]*U. Lille, INSERM, IPL, EGID-U1011*
[2]*U. Lille, US41*
[3]*U.Paris Cité, INEM, INSERM-U1151*

Despite decades of lipid-lowering drug use, prevention strategies and efforts in research, cardiovascular diseases, mainly caused by atherosclerosis, are still the leading cause of death worldwide, especially in diabetic patients. New therapies are then mandatory to reduce the residual cardiovascular risk and to prevent atherothrombotic events.

Atherosclerosis is a chronic inflammatory disease of the vascular wall triggered by low-density lipoprotein internalization within the subendothelial space. More than the obstruction of the arterial lumen, instability, and rupture of the plaque are now recognized as the most deleterious events. Among processes triggering plaque instability, intraplaque neovascularization accelerates plaque progression, induces plaque rupture, and attenuates statin benefits in humans.

Combining a multi-omics approach on $CD14^+$ cells from high and low-cardiovascular-risk diabetic patients with laser capture microdissection experiments in human endarterectomies, we determined that low expression of *REV-ERBα* is associated with advanced and vascularized atherosclerotic plaques in humans. In mice, global Rev-erbα knock-out accelerates plaque progression in old *LDLr*$^{-/-}$ mice fed a chow diet. Using whole organ imaging, we observed that 18-month-old *LDLr*$^{-/-}$*Rev-erbα*$^{-/-}$ mice display more developed intraplaque neovessels compared to controls in the brachiocephalic artery. Intriguingly, we also identified isolated vascular structures in plaque suggesting a vasculogenic primitive network. Accordingly, *Rev-erbα* deficiency is associated with an increase of intraplaque endothelial cell and endothelial progenitor cell contents, as well as induction of the pro-angiogenic program and the expression of genes involved in endothelial progenitor cell recruitment and tip-stalk cell selection. Finally, *Rev-erbα* deletion promotes VEGF-dependent angiogenesis in ex vivo aortic ring assays while accelerating the post-natal development of the retinal vascular plexus *in vivo*.

In conclusion, we identified the nuclear receptor Rev-erb-α as a putative angiogenic inhibitor of intraplaque neovascularization in human and mouse plaques as well as in endothelial cells *in vitro*.

Lucie BERNARD

The ubiquitin-like modifier FAT10 regulates the senescence of hepatocytes during MASH progression

L.Bernard[*1], L. Clavreul[*1], N. Hennuyer[1], A. Cotte[1], C. Bourouh[1], C. Devos[1], F. Glacial[1], S. Raab[1], A. Helleboid[1], E. Vallez[1], E. Dorchies[1], C. Gheeraert[1], B. Derudas[1], J. Haas[1], V. Gnemmi[2], S. M. Francque[3], L. Van Gaal[3], A. Verrijken[3], A. Pourtier[3], G. Lassailly[5], C. Abbadie[4] , B. Staels[1], R. Paumelle[1]

**Co-firsts*
[1]*U1011-EGID France*
[2]*Pathology Department, Lille University Hospital, France*
[3]*University of Antwerp, Belgium*
[4]*UMR9020 U1277 CANTHER Lille, France*
[5]*LIRIC U995 Université de Lille, France*

The accumulation of senescent hepatocytes has been identified as a key factor in Metabolic-dysfunction Associated Steatotic Liver Diseases (MASLD) progression, from simple steatosis to Metabolic-dysfunction Associated Steatohepatitis (MASH), up to the development of cirrhosis and Hepatocellular Carcinoma (HCC). But the mechanisms and actors participating to this phenomenon are still poorly described. Here, we identify the Human Leukocyte Antigen-F Adjacent Transcript 10 (FAT10), also called Ubiquitin D (UBD), as a regulator of the senescence in hepatocytes.

In MASH patients and MASH mice hepatocytes, FAT10 is upregulated and correlates positively with senescence markers. Moreover, FAT10 is induced as a SASP factor in senescent hepatocytes in vitro, and its downregulation leads to a greater induction of DNA damage response and to a boosted lipid metabolism, resulting in a better induction and propagation of the senescence. Finally, FAT10 chronic overexpression in senescent hepatocytes leads to a loss of the senescent status, by a promotion of senescence escape.

In summary, we demonstrate that FAT10 is induced in MASH and senescent hepatocytes, supporting a loss of senescent status. This senescence escape under the control of FAT10 could be one of the key mechanisms implicated in MASH progression up to HCC.

Chloé BLONDEL

Hypothalamic FXR: a new actor of brain insulin signaling impacting peripheral glucose and lipid metabolisms?

Chloé Blondel[1], Cyril Bourouh[1], Benjamin Deckmyn[1,2], Emilie Nicolas[1], Emilie Dorchies[1], Emmanuelle Vallez[1], Emilie Caron[3], Bart Staels[1], Kadiombo Bantubungi[1]

[1]Univ. Lille, Inserm, CHU Lille, Institut Pasteur de Lille, U1011-EGID, Lille, France
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[3]Inserm UMR-S 1172, Lille, France.

Expressed in different peripheral organs, the bile acid (BA) nuclear receptor FXR is known to mediate the BA effects on glucose metabolism, hence impacting type 2 diabetes. For instance, FXR activation, through gene expression, regulates hepatic glucose production (HGP). In addition to its indisputable action on peripheral organs, insulin also acts on several hypothalamic neuronal circuits to control peripheral glucose and lipid homeostasis. Indeed, hypothalamic insulin signaling is necessary for optimal suppression of HGP by acting on vagal innervation of the liver and also enables an increase of lipogenesis and a decrease of lipolysis in white adipose tissue. With our previous data concerning the FXR hypothalamic expression and its activation on brown adipose tissue function, we hypothesize a new role of FXR in hypothalamic insulin signaling through which it impacts peripheral glucose and lipid metabolisms. Using a conditional hypothalamic FXR deletion model (FXR-MBH-KO) by the Cre-Lox technology, we will determine the impact of brain FXR deletion on hypothalamic insulin signaling, energy homeostasis, and endogenous glucose production. Our preliminary results suggest that the FXR hypothalamic invalidation induces an enhancement of hypothalamic insulin signaling resulting in increased inhibition of HPG, as well as increased lipogenesis in brown and white adipose tissues. In conclusion, our data will provide new insights into the control of peripheral glucose metabolism by brain FXR, which could shed light on new pathophysiological mechanisms of type 2 diabetes.

Acknowledgments: UMR1011 Team 1, PLBS UAR2014-US41, Hauts-de-France region, EGID.

Cyril BOUROUH

The ubiquitin-like modifier FAT10 is induced in MASLD and impairs the lipid-regulatory activity of PPARα

Ludivine Clavreul[1*] and Lucie Bernard[1*], Alexia K. Cotte[1], Nathalie Hennuyer[1], **Cyril Bourouh[1]**, Claire Devos[1], Bart Staels[1+] and Réjane Paumelle[1]

[1]University of Lille, Inserm, CHU Lille, Institut Pasteur de Lille, U1011, EGID, Boulevard du Professeur Jules Leclercq 59045 Lille France
**Contributed equally to this work*

Background and Aims: Peroxisome proliferator-activated receptor α (PPARα) is a key regulator of hepatic lipid metabolism and therefore a promising therapeutic target against Metabolic-dysfunction Associated Steatotic Liver Diseases (MASLD). However, its expression and activity decrease during disease progression and several of its agonists did not achieve sufficient efficiency in clinical trials with, surprisingly, a lack of steatosis improvement. Here, we identified the Human leukocyte antigen-F Adjacent Transcript 10 (FAT10) as an inhibitor of PPARα lipid metabolic activity during MASLD progression.

A**pproach and Results:** *In vivo*, the expression of FAT10 is upregulated in human and murine MASLD livers upon disease progression and correlates negatively with PPARα expression. The increase of FAT10 occurs in hepatocytes in which both proteins interact. FAT10 silencing *in vitro* in hepatocytes increases PPARα target gene expression, promotes fatty acid oxidation and decreases intra-cellular lipid droplet content. In line, Fat10 overexpression in hepatocytes *in vivo* inhibits the lipid regulatory activity of PPARα in response to fasting and agonist treatment in conditions of physiological and pathological hepatic lipid overload.

Conclusions: FAT10 is induced during MASLD development and interacts with PPARα resulting in a decreased lipid metabolic response of PPARα to fasting or agonist treatment. Inhibition of the FAT10-PPARα interaction may provide a means to design potential therapeutic strategies against MASLD.

Thais CARBINATTI

ChREBP-FGF21 axis regulates insulin sensitivity via an inter-organ dialogue

Thais Carbinatti[1], Raphael Denis[1], Véronique Fauveau[1], Dominique Langin[2], Hervé Guillou[3], Fadila Benhamed[1], Catherine Postic[1]

[1]*Université de Paris, Institut Cochin, CNRS, Inserm, Paris*
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The transcription factor Carbohydrate Responsive Element Binding Protein (ChREBP) is now recognized as a central metabolic coordinator with implications in health and metabolic diseases, including type 2 diabetes (T2D) and Non-Alcoholic fatty Liver disease (NAFLD). Glucose-induced activation of ChREBP allows its nuclear translocation and its binding to a conserved sequence (ChoRE) present on the promoters of target genes involved in the control of hepatic metabolism including fatty acid synthesis and production of Fibroblast Growth factor 21 (FGF21), a hepatokine with beneficial effects on energy homeostasis and insulin sensitivity. Data from our team reveal that mice globally deficient for ChREBP (*Chrebp*$^{-/-}$ mice) are severely insulin resistant and exhibit a paralleled reduced production of FGF21 in response to a fasting *in vivo*. Analysis of the insulin signaling pathway revealed decreased insulin-mediated activation of Akt in brown and white adipose tissue (WAT), liver and muscle, crucial organs for metabolic homeostasis. We also observed that *Chrebp*$^{-/-}$ mice exhibit a significant decrease in WAT mass and in circulating concentrations of β-hydroxybutyrate (β-OH), a ketone body that plays a role on chromatin accessibility modulation. This led us to perform ChIP-qPCR analysis, which revealed that *Fgf21* promoter accessibility was significantly reduced in the liver of *Chrebp*$^{-/-}$ mice. Since, β-OH production is WAT dependent through the release of free fatty acids, our data suggest the involvement of an inter-organ cross-talk between WAT and liver for the regulation of FGF21 by ChREBP. In order to re-induce β-OH levels, *in vivo* experiments were carried out in *Chrebp*$^{-/-}$ mice subjected for 2 weeks to a ketogenic diet (KD). Our results showed that KD is able to correct insulin resistance in *Chrebp*$^{-/-}$ mice and correlates with a significant re-increase in circulating FGF21 concentrations. This was linked to increased activation of the insulin-Akt pathway in WAT, which also ameliorated the profile of adipokines expression. Interestingly, administration of FGF21 adenovirus (10 days treatment) provided similar results suggesting that the positive effect of the ketogenic diet in restoring insulin sensitivity in *Chrebp*$^{-/-}$ mice is mediated by FGF21. Our study reveals the importance of the ChREBP-FGF21 axis in the control of insulin sensitivity.

Inés CASTRO-DIONICIO

Automation of Untargeted Metabolomics Analysis in Cohort Samples: Adapting Standardized Sample Processing Methods

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Metabolomics plays a pivotal role in deciphering the intricate web of molecular interactions within biological systems. In this context, the automation of sample processing and analysis procedures has emerged as a vital advancement, streamlining processes and enhancing the throughput of metabolomic studies[1]. In this study, we adapted two methods[2] for the processing of commercial human serum samples for compatibility with an automated liquid handling robot. Standardized methods are tailored to microtube work volumes and are designed for the extraction and reconstitution of hydrophilic and hydrophobic compounds. To increase throughput[3], we adapted sample-solvent ratio and volumes to be compatible with 96-well plates while maintaining the initial sample-solvent ratio. The samples were analyzed using ultra-performance liquid chromatography coupled with HRMS (Vanquish Duo chromatography coupled to Orbitrap mass spectrometer, ThermoFisher Scientific) on the Integrative-Metabolome Phenotyping AnalytiCs for Translational and Precision Medicine (IMPACT-PM) platform in Lille. Two separate chromatographic methods specific for each sample extraction method utilized the following conditions: an LC–MS/MS method for hydrophilic compounds in positive and negative modes; and an LC–MS/MS method for hydrophobic compounds in positive and negative modes. The Orbitrap mass analyzer operated at 60 000 mass resolution with the methods alternating between full scan MS and data-dependent MS/MS scans. The scan range covered 67–1000 *m/z*. The adapted methods showcased the same sensitivity and reproducibility compared to the standardized processing methods. These results ease the path to adopting automated handling systems and prepare our platform for the demands of large-scale cohort metabolomics studies while maintaining a wide coverage of small metabolites.

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Ludovica COTELLESSA

Reproductive and metabolic improvement of polycystic ovary syndrome through normalization of anti-Müllerian hormone signaling over minipuberty in mice

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Polycystic Ovary Syndrome (PCOS) is the most common endocrine disorder affecting up to 18% of women of reproductive age worldwide. The condition was originally classified as a reproductive disorder, being the most common cause of anovulatory infertility in women. Today it is well known that PCOS has other long-term health repercussions, including obesity, type-2 diabetes, and increased risk for cardiovascular disease. Despite the detrimental consequences on women's health, PCOS has no cure, thus the development of treatment options is an urgent need. It is well known that hyperandrogenism together with high circulating anti-Müllerian hormone (AMH) levels are common features in PCOS women. Previous studies from our group have shown that exposure to high AMH levels in utero leads to PCOS-like traits in adult female offspring. However, whether a critical temporal window of susceptibility of PCOS exists also during postnatal life is ill-defined. Clinical studies have shown that daughters of women with PCOS display elevated AMH levels during mini-puberty. Here we provide evidence that PCOS-like female mice have also higher circulating levels of AMH during minipuberty than in control animals. We thus wondered whether we could prevent the manifestation of PCOS-like traits in adulthood by lowering the aberrant AMH-signaling during mini-puberty, using a novel molecular approach that we have designed. We functionally validated this approach respectively in vitro, treating a mouse ovarian cell line, ex vivo, performing electrophysiology in GnRH neurons of PCOS-like and control animals, and in vivo, leveraging our preclinical PCOS model. The latter investigation showed that counteracting the aberrant AMH signaling in PCOS mice during minipuberty prevents the manifestation of PCOS-like reproductive defects (oligo-anovulation), and metabolic alterations, including glucose intolerance, increased adiposity and it normalizes the deficit of orexigenic pro-opiomelanocortin (pomc) neurons in the hypothalamus of PCOS animals.

Overall, our study points to aberrant AMH signaling during minipuberty as a potential trigger predisposing the onset of PCOS in adulthood and it opens new therapeutic avenues for this syndrome.

Arnaud DANCE

Exploring the Role of Purinergic Receptor P2RY1 in Type 2 Diabetes Risk and Pathophysiology: Insights from Human Functional Genomics

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Genome-wide association studies (GWAS) have shown that P2RY1 locus is associated with type 2 diabetes (T2D) risk. P2RY1 is a G-protein coupled receptor (GPCR) activated by ADP, which is highly expressed in pancreatic islets, particularly in β cells where, ATP/ADP stimulate insulin secretion by activating the ATP-dependent potassium channels. Through functional genetics, we aimed to analyze the putative contributions of genetic variants of P2RY1 to T2D risk. We then characterized the effect of P2RY1 in insulin secretion from pancreatic beta cells.

P2YR1 was sequenced in 9,266 adults including cases with T2D and normal glucose controls. To assess the functional effect of each identified variant, we performed luciferase assays (NFAT-RE system) on HEK293 cells overexpressing each variant, in response to P2YR1 activation (using a specific agonist of P2RY1, MRS2365). We also performed expression quantitative trait loci (eQTL) analysis in 103 donors. In human pancreatic β cells (EndoCBH5), we performed glucose-stimulated insulin secretion (GSIS) assays coupled to P2YR1 activation by MRS2365 and RNA-seq to decipher signaling pathways downstream P2RY1.

We identified 22 rare missense variants in P2RY1. Our in vitro analyses highlighted 7 loss-of-function mutations. 87% of the mutation carriers presented with T2D. Expression QTL analyses showed that a block of SNP located in an enhancer of human islets was significantly associated with increased islet expression of P2RY1 and decreased T2D risk while another block of SNPs was significantly associated with decreased expression of P2RY1 in human islets and increased T2D risk. In EndoCBH5, GSIS assay showed that the P2RY1 specific agonist increased insulin secretion. RNA-seq analyses highlighted TXNIP (Known to be upregulated during beta cell dysfunction) as one of the main transcriptomic markers of insulin secretion triggered by P2RY1 agonist.

Our findings suggest that P2RY1 inherited or acquired dysfunction increases T2D risk and that P2RY1 activation stimulates insulin secretion. Selective P2RY1 agonists, impermeable to the blood-brain barrier, could serve as potential insulin secretagogues.

Maxime DESLANDE

Hippurate, a host-gut microbiome co-metabolite, modulates hepatocyte metabolome

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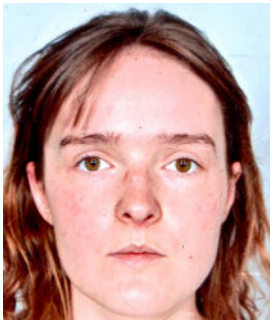
The gut microbiota has multiple mutualistic interactions with their host, including through the production of biologically-active metabolites such as short-chain fatty acids, bile acids, aromatic amino acid metabolites such as imidazoles, indoles and cresols. The list is growing and since 2006, hippurate has been described as one of the new host-beneficial metabolites. Hippurate is the product of a phase 2 conjugation of benzoate, synthesized by bacterial phenylpropanoid metabolism with glycine, and is subsequently excreted by kidneys. Hippurate associates with microbial gene richness and with metabolic health in obese individuals. It improves glucose tolerance in mice fed with high-fat diet (HFD), while stimulating insulin production and β-cells proliferation. Hippurate is also negatively correlated to hepatic steatosis. We hypothesised that in lipotoxicity conditions observed obesity and diabetes, hippurate participates in maintaining metabolic homeostasis. We set up a UPLC-HRMS/MS metabolomic approach and demonstrated that i) hippurate enters immortalised human hepatocytes (IHH) and ii) impacts the metabolome of hepatocytes towards an increase in lipid catabolism and inhibition of pro-inflammatory pathways such as prostaglandins under palmitate stress conditions. These results also show a decrease in triglyceride storage and improved viability of hippurate-treated cells. Hippurate acts as a modulator of metabolic homeostasis at the cellular level, leading to a better response to lipotoxic stress and strengthening the relevance of its study in the context of metabolic diseases.



Audrey DUPRÉ

Adipocyte specific RORa invalidation favors adipocyte hypertrophy in obesogenic conditions

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The obesity epidemic is a major public health problem. During obesity, excess energy is stored in white adipose tissue (WAT) which expands by increasing adipocyte size (hypertrophy) and/or number (hyperplasia). While hypertrophy favors metabolic complications, hyperplasia seems protective. Heterogeneous responses are also observed depending WAT depots (subcutaneous WAT (scWAT) vs visceral WAT (vWAT). Identifying the factors controlling WAT plasticity is crucial to limit the deleterious effects of obesity. The nuclear receptor ROR alpha (RORa) regulates glucid and lipid metabolism but its role in mature adipocytes is unclear.
Here, we developed mice in which RORa is specifically invalidated in mature adipocyte (*Rora*^{AdKO}), and compared them to their *RORa*$^{fl/fl}$ littermates after low fat (LF) or an obesity-induced high fat (HF) feeding for 16 weeks. HF-fed *RORa*^{AdKO} mice displayed increased scWAT without change in vWAT. This scWAT expansion is characterized with increased expression of hypertrophic markers (*Leptin, Mest*), collagen genes (*Col5a3, Col6a2*) and altered expression of lipid metabolism genes (*Apoe, Pla2g5*). Histological analyzes confirmed scWAT hypertrophy in HF-fed *RORa*^{AdKO} mice. Overall, our results indicate that adipocyte specific RORa invalidation impacts adipocyte in a depot-specific manner, favoring hypertrophy and adipocyte fat storage in scWAT during obesity. Further studies are needed to dissect the mechanisms of Rora-induced hypertrophy and its metabolic consequences.

Lise FERRI

Role of the nuclear receptor Rev-erb-α in vascular calcification

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Introduction
Vascular calcification participates to arterial stiffness and represent a main cause of atheroma plaque rupture leading to myocardial infarct. The nuclear receptor Rev-erbα displays anti-atherogenic properties by improving the lipoprotein metabolism and promoting anti-inflammatory activities. Transcriptomic analysis of Rev-erbα deficient pro-atherogenic mice led us to hypothesize that Rev-erbα impairs arterial calcification processes.

Objectives
To identify the role of Rev-erbα in vascular calcification in human, we compared the expression of REV-ERBα in low and high cardiovascular risk diabetic patients and in calcified versus non-calcified zone of human endarterectomies (n=14-21 per group). We then assessed the molecular and cellular effect of Rev-erbα deficiency on vascular calcification *in vivo* in aged LDLr$^{-/-}$ Rev-erb-α$^{-/-}$ and LDLr$^{-/-}$ Reverb-α$^{+/+}$ mice fed a chow diet (n=8-10), as well as in vitro in primary aortic smooth muscle cell (SMC) (n=3-6).

Results
In human, Rev-erbα expression is higher in diabetic patients with a low calcium score (CAC) compared to high CAC patients, and in non-calcified region compared to calcified zones in human endarterectomies, then suggesting a protective role of Rev-erbα against vascular calcification. We then confirmed in mice that Rev-erbα deficiency promotes atherogenesis, increases calcium deposition and induces differentiation of SMC toward osteoblast-like (OSB) cells. *In vitro*, Rev-erbα also controls the dedifferentiation of SMCs into OSB cells in pro-inflammatory conditions by regulating the expression of key ostogenic factors.

Conclusion
In conclusion, Rev-erbα is a key regulator of SMC-driven vascular calcification *in vivo* and *in vitro*. Rev-erbα then represents a novel therapeutic target to reduce the residual cardiovascular risk.

Key words
Vascular Calcification, Smooth Muscle Cell, Atherosclerosis, Nuclear Receptors.

Lise FOLON

Pathogenic, total loss-of-function *DYRK1B* variants cause monogenic obesity associated with type 2 diabetes

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Obesity and type 2 diabetes are complex disorders with a strong genetic basis. Their monogenic forms, driven by a rare singular mutation with a strong effect on disease onset, are generally severe and manifest earlier in life than common forms. Pathogenic rare variants of *DYRK1B* (*dual-specificity tyrosine phosphorylation-regulated kinase 1B*) have been identified in several patients with metabolic syndrome that includes central obesity, type 2 diabetes, early-onset coronary disease and hypertension. Due to the limited number of conducted studies, the broader impact of *DYRK1B* variants has not been assessed on a large scale. In this study, we conducted an extensive functional genomics investigation focusing on rare variants of *DYRK1B*.

DYRK1B was sequenced in 9,353 individuals (including obese and/or diabetic patients, and controls) by next-generation sequencing (Roche/Illumina). The detected mutations were created by mutagenesis and inserted into plasmids. The effect of each *DYRK1B* variant on the Wnt signalling pathway was evaluated through in vitro assays. Variant pathogenicity was determined based on functional tests, in addition to other criteria from the American College of Medical Genetics and Genomics. Subsequently, the effect of pathogenic or likely pathogenic (P/LP) variants on metabolic traits was assessed using association analyses.

We identified 65 rare, heterozygous *DYRK1B* variants that showed no association with the risk of obesity or type 2 diabetes. Following *in vitro* analyses, we pinpointed 20 P/LP variants, including six variants that demonstrated a fully inhibitory effect (P/LP-full) on DYRK1B activity. P/LP and P/LP-full *DYRK1B* variants were associated with increased body mass index and a higher risk of obesity; however, the impact was notably more pronounced for the P/LP-full variants (π=8.0 and OR=7.9). Furthermore, P/LP-full variants were associated with higher fasting glucose levels and an elevated risk of type 2 diabetes (π=2.9 and OR=4.8), while P/LP variants had no effect on glucose homeostasis.

These results highlight that complete loss-of-function P/LP *DYRK1B* variants lead to a monogenic form of obesity associated with type 2 diabetes. This study underscores the significance of performing *in vitro* functional assessments to accurately determine the tangible effects of *DYRK1B* variants.

Vance Gao

Glutamine deficiency and fatty acids induce high IL-23 production in TLR-activated dendritic cells

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We previously demonstrated that fatty acids induce a distinct immunometabolic profile in TLR-activated dendritic cells (DCs), characterized by very high IL-23 production and reduced glycolysis. Now, transcriptomic evidence leads us to examine glutamine and related amino acids (AAs) in DC immunometabolism. We show that glutamine deprivation increases IL-23 production in DCs, phenocopying the effect of fatty acid treatment. siRNA and supplementation experiments suggest that deficiency of glutamate and aspartate are also part of the mechanism. We hypothesize that AA deficiency contributes to the palmitate-induced increase of IL-23, since palmitate alters the expression of many key AA enzymes (Gclc, Asns, Got1) and transporters (Slc1a5, Slc25a22, Slc38a1). Palmitate also upregulates AA-consuming pathways, such as hexosamine and glutathione synthesis. Fatty acid and amino acid signals may further converge at the integrated stress response. These results shed new light on the complex role of amino acid metabolism in the tuning of innate immune responses

Valentine Guinot

Bile acid pool composition impacts invariant natural killer T cells in mouse liver

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Bile acids (BA), synthesized in the liver from cholesterol, facilitate intestinal lipid absorption and act as signaling molecules *via* their binding to receptors such as TGR5, FXR and VDR which, in turn, modulate glucose, lipid as well as BA metabolism. Dysregulation of metabolism induces the onset of pathological contexts such as obesity and Type 2 diabetes, main risk factors for metabolic-associated steatohepatitis also characterized by a severe immuno-inflammation. Recently, BA were shown to directly affect immune cells via both bile acid receptors-dependent and -independent mechanisms. Species-specific differences in BA metabolism complicate the translation of experimental results obtained in mice to human pathology. Murine hepatocytes express CYP2C70 enzyme reported as converting the primary hydrophobic chenodeoxycholic acid into hydrophilic α-β muricholic acids (MCAs). The deletion of *Cyp2c70* gene resulted in a "humanized" mouse model presenting a more hydrophobic bile acid pool due to the absence of MCAs and a spontaneous liver disease progressing to bridging fibrosis in female mice fully reversed by treatment with ursodeoxycholic acid (UDCA). The mechanisms by which an imbalanced BA pool may influence the hepatic immune compartment in liver pathologies remain to be investigated.

For this purpose, we firstly characterized the hepatic immune compartment of young adult male and female *Cyp2c70*$^{-/-}$ mice in comparison with littermate controls. Our immune profiling showed a robust reduction in the number of liver resident invariant natural killer T cells (iNKTs) and altered dendritic cell subsets, which further revealed an activated and inflammatory profile in both male and female *Cyp2c70*$^{-/-}$ mice. On the basis of iNKTs capacity to respond to CD1d-restricted glycolipid antigen presentation, we observed significant changes in transcriptomic profiles and CD1d gene expression in antigen presenting cells, in particular, Kupffer cells and hepatocytes, which specifically express *Cyp2c70*. Our analysis of the lipid content and functional study of the antigenic presentation capacity of these two cell subsets are aimed at identifying the direct lipid or cellular origin of iNKTs activation. In parallel, we investigate the efficacy of UDCA treatment in restoring immune alterations in order to clarify the direct/indirect role of bile acid pool hydrophobicity on iNKTs.

Doriane HENRY

Hepatocyte PPARα is essential for pemafibrate-induced beneficial effects on NAFLD, dyslipidemia and atherosclerosis

Doriane Henry[1], Eric Baugé[1], Nathalie Hennuyer[1], Bruno Derudas[1], Emmanuelle Vallez[1], Audrey Deprince[1], Joel T Haas[1], Philippe Lefebvre[1], Bart Staels[1], Fanny Lalloyer[1]

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Background and aims: Due to its major roles in lipid metabolism (transactivation) and inflammation (transrepression), peroxisome proliferator-activated receptor (PPAR) modulation is increasingly being considered as an important therapeutic option for non-alcoholic fatty liver disease (NAFLD). Besides, pemafibrate, a novel highly potent and selective PPARα agonist, can recover liver function in NAFLD patients, but the mechanisms are not understood. The aim of our study was to evaluate the importance of selective hepatocyte PPARα activation (transactivation vs transrepression) in the effects of pemafibrate on NAFLD, dyslipidemia and atherosclerosis, in the low-density lipoprotein receptor knockout (*LDLr*$^{-/-}$) mouse model.

Methods: *LDLr*$^{-/-}$ *PPARα*$^{+/+}$ and *PPARα*$^{-/-}$ mice were fed a western diet containing 0.2% cholesterol supplemented or not with pemafibrate for 8 weeks. To determine the role of hepatocyte *PPARα*, *LDLr*$^{-/-}$ *PPARα*$^{-/-}$ mice were injected with an AAV8-TBG-*PPARα*^{WT} (to restore both transactivation and transrepression PPARα activities), with an AAV8-TBG-*PPARα*^{DISS} (PPARα mutant only exerting hepatocyte transrepression PPARα activity) or with an AAV8-TBG-GFP (control), and subsequently challenged with the same diets.

Results: Pemafibrate reduced the steatosis score and number of CD68 (macrophage marker)-positive cells in the liver, improved atherogenic dyslipidemia and decreased atherosclerosis in *LDLr*$^{-/-}$ *PPARα*$^{+/+}$, but not in *LDLr*$^{-/-}$ *PPARα*$^{-/-}$ mice. Interestingly, pemafibrate strongly decreased the hepatic steatosis score in AAV8-*PPARα*^{WT} mice, but to a lesser extent in AAV8-*PPARα*^{DISS} mice, and significantly decreased CD68-positive cells in livers of both AAV8-*PPARα*^{WT} and AAV8-*PPARα*^{DISS} mice. Plasma triglyceride and cholesterol levels were reduced by pemafibrate in AAV8-TBG-*PPARα*^{WT}, but not in AAV8-TBG-*PPARα*^{DISS} mice, suggesting that hepatocyte PPARα transactivation activity is required to improve atherogenic dyslipidemia. Surprisingly, pemafibrate reduced atherosclerotic plaques to a similar extent in AAV8-TBG-*PPARα*^{WT} and AAV8-TBG-*PPARα*^{DISS} mice, showing that pemafibrate decreases atherosclerosis in a distal manner through hepatocyte PPARα transrepression activity.

Conclusion: We here demonstrate that hepatocyte PPARα is the key driver of the beneficial effects of pemafibrate on NAFLD, dyslipidemia, and atherosclerosis development in the *LDLr*$^{-/-}$ mouse model.

KEYWORDS: PPARA, PEMAFIBRATE, NAFLD, DYSLIPIDEMIA, ATHEROSCLEROSIS

Ghania KARA-ALI

Dissecting adipose tissue cellular heterogeneity during weight loss

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The adipose tissue (AT) remodeling process at a cellular level in a post-obesity weight loss context is poorly understood. To better characterize the underlying mechanisms, we established a mice model of post-obesity weight loss by switching from an obesogenic to a standard chow diet. We analyzed the metabolic and tissular changes in both depots (inguinal subcutaneous AT, iAT, and epididymal visceral AT, eAT) using a single nuclei RNAseq approach. The diet switch decreased body weight by 20% and the AT adiposity by 25 to 40% after 4 weeks. At the adipocyte level, the eAT is characterized by 4 subpopulations specialized mainly in immunity, extracellular matrice remodeling (ECM), insulin signaling, and lipid handling. While the iAT is characterized by transcriptionally different subpopulations, however, functionally, they are also implicated in ECM remodeling, insulin response, and lipid metabolism. During obesity, insulin signaling subpopulation proportion is strongly decreased in both depots iAT and eAT and only slightly restored after 4 weeks of weight loss. Conversely, the ECM subpopulation increases, and proportions don't vary even after 4 weeks of weight loss. Thus, we report, in our mice model, a dynamic remodeling of adipocyte subpopulations in obesity and weight loss in each depot, contributing to a better understanding of adaptative and pathological responses of AT.

Camilla KLAIMI

Understanding the effect of intracellular metabolic reprogramming of dendritic cells on MASH development

Camilla Klaimi[1], Pauline Jacquemain[1], Kevin Ory[1], Charlène Pourpe[1], Arnaud Carrier[2], Alexandre Pelletier[2], Fabien Delahaye[2], David Dombrowicz[1], Bart Staels[1], Delphine Eberlé[1], Joel T. Haas[1]

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Context: Metabolic-Associated Steatotic Liver Disease (MASLD) is the most common chronic liver disease worldwide. It is associated with triglyceride (TG) accumulation in hepatocytes leading to inflammation and can progress to Metabolic-Associated Steatohepatitis (MASH). We previously found that altered hepatic conventional dendritic cell (cDC) populations are associated with MASH in humans and mice with the ratio of cDC1/cDC2 being decreased. Results in animal models indicate that total cDC ablation can accelerate MASH development, suggesting the cDC phenotype in MASLD can impact disease progression. Our preliminary results revealed higher expression of peroxisome proliferator activated receptor δ (PPARδ), a transcription factor activated by fatty acids, in bone-marrow-derived DCs activated by TLR agonists in a high free fatty acid environment, as found in the liver in MASH. We thus hypothesized that cDC PPARδ may control the cDC phenotype in MASH.

Objective: Identify changes in cDC gene expression in MASLD and the signals driving their expression.

Materials and methods: Single cell RNAseq (scRNAseq) was performed on sorted hepatic cDC from chow- and MASH-diet fed mice. We used PPARδ-deficient bone-marrow derived DC (BMDC) that can be differentiated into cDC2-like cells submitted to different metabolic and immune treatments. We measured gene expression using RT-qPCR on purified RNA from BMDC cultures.

Results: Our scRNAseq results identified a MASH-associated cluster of cDC with markers corresponding to mature regulatory DC called (mregDC), including *Ccr7, IL12b, Ccl5* and *Ccl22*.

Discussion: Previously, we demonstrated that increased extracellular fatty acids profoundly affect DC immune function by the modification of their intracellular metabolism. Moreover, we observed a higher expression of mregDC markers in *wild-type* BMDCs treated with LPS and LPS+Palmitate and a reduced expression of these markers in PPARδ-deficient BMDCs in the same conditions.

Conclusion & Perspectives: Together, these results demonstrated that PPARδ has a pro-inflammatory activity especially in cDC2 that can support the expression of some genes in the mregDC program Further investigation will seek to identify metabolic pathways required to induce the mregDC gene program in MASLD.

Fanny LALLOYER

A new relevant mouse model of progressive non-alcoholic fatty liver disease (NAFLD) and atherosclerosis development

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Background and aims: Cardiovascular diseases (CVD) are the most frequent causes of death in non-alcoholic fatty liver disease (NAFLD) patients, but links between NAFLD and CVD remain unclear and there is a lack of pre-clinical models that combine both pathologies. Therefore, there is a rationale to develop a new relevant mouse model of progressive non-alcoholic steatohepatitis (NASH) and atherosclerosis that recapitulates human disease.

Methods: Female and male low-density lipoprotein receptor knockout (*LDLr*$^{-/-}$) mice, deficient or not for the nuclear receptor peroxisome proliferator-activated receptor-alpha (PPARα), were challenged with a chow or a high-fat (HF) diet during 12 and 18 weeks.

Results: HF diet feeding increased liver weight and injury in a time-dependent manner, with different histological characteristics during NAFLD progression according to sex and *PPARα*-deficiency. Female *LDLr*$^{-/-}$ mice under HF diet developed mostly steatosis at 12 weeks, exhibiting all characteristics of human NASH (ie steatosis, inflammation and ballooning) at 18 weeks with presence of mild fibrosis. NAFLD progression was aggravated upon deletion of *PPARα* in the *LDLr*$^{-/-}$ mice, with presence of both strong inflammation and fibrosis already after 12 weeks of HF diet. Male *LDLr*$^{-/-}$ mice developed obesity and insulin-resistance over time, and strong steatosis and ballooning already appeared after 12 weeks of HF diet, with strong inflammation and mild fibrosis at 18 weeks. Male *PPARα*-deficient *LDLr*$^{-/-}$ mice were protected from obesity, but developed stronger NASH with already strong steatosis, inflammation and ballooning, with mild fibrosis at 12 weeks. In addition, HF diet feeding led to progressive atherosclerosis development in *LDLr*$^{-/-}$ mice, which was more severe in female compared to male mice, and with *PPARα*-deficiency.

Conclusion: We describe a new relevant mouse model of progressive NAFLD that recapitulates all histological characteristics of human NASH, along with progressive atherosclerosis development, in a relatively short time period (12-18 weeks). Histological and metabolic specificities associated to sex and *PPARα*-deficiency will help to explore mechanisms linking NAFLD to atherosclerosis progression and can be relevant to test new cardiometabolic drugs on NASH/NAFLD and CVD risk.

Keywords : NASH, ATHEROSCLEROSIS, MOUSE MODEL

Margaux LEDUC

The effect of an obesogenic-diabetogenic diet on muscle regeneration

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Skeletal muscle is a major metabolic tissue responsible for 80% of post-prandial glucose handling. Maintaining muscle homeostasis is therefore essential. This tissue has a significant capacity to regenerate following injury or intensive exercise. Skeletal muscle regeneration involves the activation of muscle stem cells as well as a coordinated inflammatory response.

Several studies have highlighted defects in muscle regeneration in obesity or type 2 diabetes. In this context, the function of muscle stem cells is known to be impaired. However, how these metabolic conditions impact the inflammatory process associated with muscle repair is poorly understood. The aim of this study was to determine the effect of an obesogenic-diabetogenic diet on the muscle regeneration process.

Wild-type mice were fed either Chow diet (CD) or High Fat High Sugar and cholesterol diet (HFHSC) for 12 weeks before being injured with an injection of 0.8% of barium chloride (BaCl2) in the tibialis anterior (TA). First, the efficacy of the diet was validated by regular weight monitoring and metabolic tests, which revealed glucose intolerance after 10 weeks on the diet. Adipose tissue hypertrophy and a decrease in muscle mass were also observed. Histological studies of fiber size distribution and fiber size area showed no significant difference between the two groups of mice 7 days post-injury. Myosin mRNA expression analysis reveals a delayed myogenic process in obese mice compared to CD mice. Interestingly, immunophenotyping experiments performed 4 days after the injury revealed a significant reduction in the proportion of different infiltrating leukocyte populations, namely macrophages, eosinophils and T lymphocytes, in the muscle of HFHSC mice compared to CD mice.

In conclusion, we demonstrated that obesity and glucose intolerance impair the recruitment of immune cell populations into skeletal muscle after injury.

Valentin LERICQUE

Long-term GABA administration to transdifferentiate human α-cells into insulin-secreting β-cells *in vitro* and *in vivo*: heterogeneity between donors?

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Y-aminobutyric acid (GABA) converts α-cells to β-cells in both murine and human islets *in vitro* and *in vivo*. Murine studies proved controversial and only one publication confirmed with a single human islet preparation. This study aims to verify the effect of GABA in a larger number of human islet preparations and look for potential donor heterogeneity of response.

Methods: 16 human islet preparations were used. *In vitro*, 5 preparations were dissociated and cultured ± GABA at different concentrations (1µM, 100µM, 1mM) until 72 hours for a dose and time-response. 6 preparations were cultured ± GABA (100µM) in 3D in Matrigel for 15 (n=6) or 28 days (n=3). *In vivo*, 7 preparations were transplanted under the renal capsule of immunodeficient mice (n=3 control and n=3 treated per donor). GABA (20µM) was administered daily intraperitoneally for 28 days with monitoring of blood glucose, weight and human C-peptide levels. In the end, human islets were fixed, embedded in paraffin and immunofluorescence labeling was performed. Counting and quantification were assessed in *ImageJ*.

Results: After 72 hours, GABA (100µM) induces a slight increase in nucleocytoplasmic translocation of ARX synonymous of a potential transdifferentiation, donor-dependent (2.68 ± 0.56% Control vs 4.65 ± 0.63% GABA; p=0.1347). A long-term GABA administration induced a moderate conversion of α-cells to β-cells: *In vitro*, the mean percentage of conversion was Δ7.26 ± 3.82% (p<0.01) after 15 days and Δ4.44 ± 1.84% (p<0.01) after 28 days. *In vivo*, the mean percentage of conversion was Δ3.17 ± 1.75% (p<0.01).
A heterogeneity between donors was observed: 4/6 of responders after 15 days, 3/3 after 28 days *in vitro*, and 4/7 of responders *in vivo*. *In vivo*, GABA did not induce α-cell neogenesis after a first transdifferentiation cycle (1.39 ± 0.77% Control vs 0.64 ± 0.18 % GABA), but significant proportions of PDX1+ duct cells were observed whatever the conditions (79.42 ± 2.30%). At one month follow-up, GABA did not change blood glucose, weight and human C-peptide levels compared to control mice.

Conclusions: The effect of GABA on the transdifferentiation of human α-cells to β-cells appears moderate and heterogeneous between donors. Special transcriptomics studies will enable us to understand the mechanism of action of GABA and to see how transdifferentiation changes evolve during treatment.

Laurent L'HOMME

Adipose tissue macrophage infiltration and hepatocyte stress increase GDF-15 during the sequential development of obesity, type 2 diabetes and MASH

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Growth differentiation factor-15 (*GDF-15*) and its receptor GFRAL are key players in the regulation of food intake and energy expenditure. Despite its protective effect against weight gain, circulating *GDF-15* levels increase with obesity and metabolic dysfunction-associated steatotic liver disease (MASLD), but the underlying mechanism remains poorly defined.

Using mouse models of obesity and MASLD, and biopsies from carefully-characterized patients regarding obesity, type 2 diabetes (T2D) and MASLD status, we identify adipose tissue (AT) as the key source of *GDF-15* at the onset of obesity and T2D, followed by liver during the progression towards metabolic dysfunction-associated steatohepatitis (MASH). Obesity and T2D increase *GDF15* expression in AT through the accumulation of macrophages, which are the main immune cells expressing *GDF15*. Inactivation of *GDF15* in macrophages reduces plasma *GDF-15* concentrations and exacerbates obesity in mice. During MASH development, *GDF15* expression additionally increases in hepatocytes through stress-induced TFEB and DDIT3 signaling.

Together, these results demonstrate a dual contribution of AT and liver to *GDF-15* production in metabolic diseases and reveal the distinct involvement of immune and non-immune actors in this process.

Viktor LIÉNARD

Apolipoprotein F deficiency is associated with reduced mitochondrial function in hepatocytes

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Context: Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD, previously NAFLD) is the most common chronic liver disease worldwide, with its prevalence rising in concert with rates of obesity. MASLD is characterized primarily by hepatic triglyceride (TG) accumulation, called steatosis, which can progress to metabolic dysfunction-associated steatohepatitis (MASH), which combines steatosis with elements of hepatic necroinflammation. Our group recently reported that hepatic *APOF* (encoding Apolipoprotein F) mRNA expression inversely correlates with steatosis and the severity of MASLD in humans. In addition, previous studies have shown that lipoprotein-associated *ApoF* could increase mitochondrial function in brown adipocytes. Therefore, our aim is to determine if reduced *ApoF* expression contributes to progression from steatosis to MASH or associated complications.

Methods: We established a new mouse model deficient for ApoF (ApoF-KO). ApoF-KO mice and littermate controls were fed a high-fat diet supplemented with sucrose and cholesterol (HFSC diet) for 22 weeks. After sacrifice, hepatic lipids were measured and gene expression was analyzed by RNAseq. We measured mitochondrial function in primary hepatocytes (PH) from chow-fed ApoF-KO mice and littermate controls with Seahorse Extracellular Flux Analyzer.

Results: No changes in body weight, fasting plasma lipids and glycemia were observed between ApoF-KO and control mice on HFSC diet. Biochemical and histological evaluation of the liver revealed no differences in TG or cholesterol content and no morphological or structural differences between genotypes. Furthermore, transcriptomic analysis revealed no differences in gene expression, together suggesting similar MASH development in ApoF-KO and littermate controls. However, PH from chow-fed ApoF-KO mice displayed decreased mitochondrial function (notably decreased spare respiratory capacity) compared to littermate controls, suggesting a role for *ApoF* in the control of hepatic mitochondrial metabolism.

Discussion: Our results suggest that *ApoF* deficiency does not accelerate MASH progression. However, ApoF depletion impacts mitochondrial function in hepatocytes. We hypothesize this may result from alterations in hepatocyte lipid trafficking. Further studies are necessary to elucidate the link between ApoF expression and mitochondrial function.

Isaline LOUVET

Recombinant PSP/reg treatment improves beta cell function in islets isolated from glucose intolerant and type 2 diabetic donors

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Background and aims: Pancreatic islet dysfunction and demise are key characteristics of type 1 diabetes (T1D), type 2 diabetes (T2D), and HNF1A-MODY. Pancreatic Stone Protein / Regenerating protein (PSP/reg) is regulated at the transcription level by the Hepatocyte Nuclear Factor family of proteins and physiologically secreted from pancreatic acinar cells. Upon focal or systemic extra-pancreatic inflammation, PSP/reg strongly increases, but is also highly elevated in the serum of T1D, T2D, and HNF1A-MODY patients. Although, PSP/reg is expressed and induced in islets of diabetic mice, the mechanisms involved remain largely unknown. Therefore, the aim of this study was to investigate the expression and regulation of PSP/reg in the human pancreas and islets isolated from donors covering a wide spectrum of metabolic disease compared to normoglycemic controls.

Material and methods: PSP/reg protein expression was analysed from human paraffin embedded pancreatic sections and isolated islets embedded in histogel by immunofluorescence. PSP/reg gene and protein expression was analysed by qPCR and Western Blotting. Perifusion techniques were used to determine glucose-stimulated-insulin-secretion (GSIS) in islets isolated from donors with glucose intolerance or T2D in response to a chronic treatment with recombinant PSP/reg (rPSP/reg).

Results: PSP/reg protein was highly enriched in the acinar cells of all donor pancreata studied. Islets embedded in histogel revealed that PSP/reg colocalized with insulin in beta cells, somatostatin in delta cells and, to a lesser extent, with glucagon in alpha cells. Of note, PSP/reg protein expression was significantly induced in islets isolated from T2D donors with obesity compared to those of obese (normoglycemic), glucose intolerant and normoglycemic donors. Moreover, the chronic treatment of rPSP/reg (24h) enhanced both, first and second phase GSIS from islets isolated from glucose intolerant donors, while only the second phase was enhanced from those of T2D donors.

Conclusions: Collectively, these data indicate that PSP/reg may play an important role in preserving beta cell function during the early stages of the disease. They also suggest that rPSP/reg could be a promising therapy for the treatment of diabetes, but further studies in diabetic mice to determine its efficacy to reduce hyperglycemia and improve islet function are warranted.

Lucas MAURIN

Biological insight of age-related dynamics of DNA methylation in pancreatic islets in the context of diabetes

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Age is the most prominent risk factor for type 2 diabetes (T2D) development. This is in-part mediated by the age-associated progressive decline of pancreatic *β*-cell function. To uncover the underlying mechanisms, we assessed the age-associated epigenetic and transcriptomic signature of pancreatic islets in 105 organ donors (age range: 22-96y, mean = 72y). DNA and RNA were extracted from pancreatic islets, and subjected to Infinium methylation EPIC array and RNA sequencing, respectively. We performed an epigenome wide association study (EWAS), adjusting for sex, BMI, and cellular composition, and identified 1,831 CpGs associated with age (bacon-corrected FDR < 0.05), of which 86% were hypermethylated and enriched in regulatory regions, including CpG islands and transcription start sites. We also confirm three previously reported dysmethylated CpG sites located in the *ELOVL2* gene, which are associated with age across many tissues and showed they may be involved in insulin secretion. We then performed a transcriptome wide association study (TWAS) and found 1,743 differentially expressed genes with age, several of which are known to be T2D risk associated genes, including transcription factors *TCF7L2* and *HNF4A*. We finally integrated transcriptomics and methylation data to identify CpGs that are likely to modify nearby genes expression (cis-window 500 kb): we found 1/ that 42 % of age-associated DNA methylation changes were associated with the expression of a nearby target gene. 2/48 % of those laters were also dysregulated with age in our TWAS analysis, including the downregulation of T2D genes involved in insulin secretion such as *ELOVL2* and *GNPNAT1*. 3/ Pathway analysis revealed an enrichment of genes in impaired glucose tolerance, and in pancreatitis, an exocrine comorbidity of T2D. In conclusion, our study identifies age-associated changes in DNA methylation with a functional impact in gene expression, bringing biological insight into age-related changes in pancreatic islets that could contribute to islet physiological decline and consequently to T2D.

Sarah MEULEBROUCK

Pathogenic mutations of *NTRK2* cause monogenic forms of obesity or metabolic syndrome depending of its isoform

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NTRK2 encodes the tyrosine kinase receptor TRKB. This protein exists under various isoforms comprising TRKB full-length (TRKB.FL) that contains a distal tyrosine kinase domain, and a shorter form (TRKB.T1) that does not. In the 2000s, it has been suggested that pathogenic mutations of *NTRK2* could cause a monogenic form of obesity but until now very few studies have been conducted regarding this gene. The aim of our current study was to investigate the contribution of rare pathogenic variants of *NTRK2* to obesity and related metabolic traits.

NTRK2 have been sequenced in 9,354 individuals from the French RaDiO study. The identified rare variants were functionally investigated *in vitro* through luciferase assays, followed by the assessment of their pathogenicity using the medical genetics criteria established by the *American College of Medical Genetics and Genomics* (ACMG). This study was then extended to exome data from more than 200,000 individuals from the UK Biobank. The phenotype of variant carriers from RaDiO and UK Biobank was finally investigated in order to determine if *NTRK2* variants are associated with obesity and/or related metabolic traits.

34 rare variants have been identified in 121 individuals from RaDiO. Based on ACMG criteria, nine of them were considered as pathogenic/likely pathogenic (P/LP). In parallel, four rare variants have been identified in 19 individuals from the UK Biobank. The phenotype analysis of variant carriers from both studies showed that in total, 80% of children carrying a P/LP variant are obese, and 81% of adults carrying a P/LP variant are overweight or obese. Furthermore, 56% of individuals carrying a P/LP variant suffer from metabolic syndrome with a significant enrichment of these variants in the proximal part of the gene, suggesting an enrichment in TRKB.T1 specifically.

In conclusion, our results suggest that *NTRK2* contributes to various phenotypes depending of the mutated isoform, as it can contribute to overweight and obesity in case of mutations of TRKB.FL that is mostly expressed in the brain, or metabolic syndrome by implying more specifically TRKB.T1 that is expressed in metabolic tissues (pancreas, adipose tissue, skeletal muscle) in addition to the brain. Metabolic syndrome could thus depend of the expression of obesity related genes in tissues contributing to this pathology.

María MORENO-LÓPEZ

Metabolic Effects of Sodium-Glucose-Co-Transporter-4 (SGLT4) Inhibition: From Mouse Studies to Clinical Translation

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Background and Objectives: Sodium-Glucose-Co-Transporter-4 (SGLT4), a member of the SGLT family with lower affinity, is instrumental in the transportation of various sugars, prioritizing mannose, glucose, fructose, 1.5AG, artificial sweeteners, and galactose. These sugars are prevalent in the Western diet (WD) and are elevated in the bloodstream of individuals afflicted by obesity, with or without type 2 diabetes (T2D). This study aims to explore the potential of SGLT4 inhibition as a transformative strategy to counter obesity and hinder the progression to T2D by reducing serum sugar levels.

Methods: We conducted a comprehensive investigation into SGLT4/Sglt4 gene expression and its relevance to obesity and T2D, using a human intestinal cohort consisting of obese patients, both with and without T2D, before and after weight-loss surgery. Additionally, we employed a novel Sglt4 knock-out (KO) mouse model.

Results: Utilizing the RNAscope technique, we observed robust mRNA expression of both *SLC5A1* (SGLT1) and *SLC5A9* (SGLT4) in the apical membrane of the intestines of obese individuals. Moreover, we identified significant *SLC5A9* mRNA expression in the exocrine pancreas, with upregulation associated with obesity and T2D.
Interestingly, *SLC5A1* mRNA levels were notably reduced (by 40%) in the intestines of obese patients, regardless of their diabetic status, while *SLC5A9* mRNA expression exhibited a more pronounced reduction (by 70%) after surgery. These changes in *SLC5A9* mRNA expression were not mirrored by alterations in the mRNA levels of *SLC5A2* (SGLT2), *SLC2A2* (GLUT2), or *SLC2A5* (GLUT5). Unlike SGLT1 and SGLT2, SGLT4 mRNA was absent in the brains of mice. Additionally, Sglt4 KO mice demonstrated resistance to obesity and T2D after just three months of a WD regimen, in contrast to wild-type (WT) mice subjected to the same diet. Islets isolated from WDfed WT mice displayed impaired glucose-stimulated insulin secretion (GSIS) compared to those from Sglt4 KO mice. The absence of SGLT4 in the brain implies that weight loss in mice does not affect feeding behavior.

Conclusion: Our findings suggest that inhibiting SGLT4 holds potential as a promising therapeutic approach for preventing metabolic disorders, such as obesity and T2D. Further research is imperative to unravel the mechanisms and clinical implications of modulating SGLT4 in the management of these conditions.

Frédérik OGER

High glucose stress leads to translation of novel short unannotated ORFs in human pancreatic β cell line

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While 20,000 genes are known in the human genome, the repertoire of proteins required for cellular functions is much larger (up to several millions). This complexity is only partly due to the different proteins produced from variations in the DNA sequences to post-translational modifications. Indeed, multi-omics approaches have shown that the protein repertoire also includes small proteins translated from short open reading frame (short ORF encoded proteins, SEPs). These SEPs are found in non-coding RNA regions such as 5'- and 3'-untranslated regions and long non-coding RNAs. The insulin secreting pancreatic β cell is a highly differentiated endocrine cell whose functional alterations triggered by systemic glucotoxicity can lead to type 2 diabetes (T2D). The objective of our study is to explore the translation process in human β cells and to identify SEPs during T2D-associated glucotoxic stress. For this purpose, we first set up an in vitro glucotoxicity model in EndoCbH5 cell line, showing that high glucose stress alters cell functionality (i.e. insulin secretion) without alter viability. Using this cell model, we next applied a multi-omics approach combining transcriptomic analysis through total RNA-seq and translatomic analysis through Ribo-seq aiming to profile ribosome-occupied RNA transcripts. Following this strategy as proxy for relative protein abundance by delivering quantitative readout of gene translation level, we not only showed that high glucose stress affects translation efficiency rather than gene expression but also identified, among other targets, a putative glucotoxic-specific SEP from Linc01574, a long non-coding RNA mainly expressed in human pancreatic islets, brain regions and pituitary. Taken together, our results suggest that the metabolic stress undergone by β cells in the context of T2D could lead to translational remodeling resulting in the expression of small unknown proteins that could play a role in the pathology.

Romina PACHECO TAPIA

Propagation of chemical families from high-confidence level metabolite identification through molecular networking in the context of microbiome research

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Host-associated samples subjected to untargeted metabolomics have provided valuable insights into how microbes influence health in a bidirectional way[1,2]. However, accurate metabolite annotation and identification remain to be a challenge[3] along with ensuring analytical reproducibility and feature coverage for large cohorts of data[4]. Human serum samples were analyzed using a UHPLC Vanquish Duo coupled to a high resolution Orbitrap Exploris™ 240 mass spectrometer with two optimized methods for polar and non-polar metabolites in negative and positive electrospray ionization mode. Intelligent data acquisition workflow was implemented in addition to the Data-Dependent Acquisition method in order to increase spectral data required for metabolite annotation. Ion Identity Molecular networking (IIMN) approach was applied using the GNPS on-line platform to expand the chemical class starting from the known metabolites, annotated with both public spectral reference library (GNPS) and an in-house spectral library, to the unknowns. The metabolome profiling of the samples provided a total of 4840 linear and reproducible features (m/z-rt pairs) detected in positive and negative mode with both LC-MS methods. Focusing on the 410 unique metabolites that were annotated, 20% correspond to a high confidence level annotation. Among them, we found lipids (45%), organic acids and derivatives (27%), organoheterocyclic compounds (10%), benzenoids (9%), organic oxygen compounds (5%) and other chemical superclasses, 38 classes and 76 subclasses. IIMN also allowed us to reduce redundancies of ion species and to expand the chemical information of the unannotated metabolites. Other metabolome mining tools, such as *in silico* approaches[5] harness advanced machine learning and predict fragmentation spectra from known structures to complement our results. This will be essential for the implementation of a reproducible workflow for untargeted LCMS analysis of biofluids in the context of metabolomics in microbiome research.

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Vincent PASCAT

Shared genetic predisposition between type 2 diabetes and blood pressure highlights common insulin resistance and central obesity pathways

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Type 2 diabetes (T2D) and elevated blood pressure (BP, including systolic, diastolic, and pulse pressure [*SBP/DBP/PP=SBP –DBP*]) are often associated, but potential shared genetic factors between T2D and BP have not been partitioned and specific pathogenetic processes leading to their comorbidity are unknown.

We first dissected the reciprocal effects of 555 T2D and 987 BP GWAS associated known variants, by building polygenic score (PGS) in an independent set of 457,386 European individuals from the UK Biobank (UKB), including 33,232 individuals with T2D. We then used a bi-directional two-sample Mendelian Randomization (MR) approach to identify which phenotype was causal on the other. We gathered Z-scores from GWAS meta-analyses on 49 endophenotypes to partition the 1,542 genetic variants into clusters of different pathophysiological processes. We analysed polygenic scores (PGS) after clustering of their effects. Finally, we assessed the causality of these relationships through a two-sample Mendelian Randomization (MR) approach.

T2D overall PGS was associated with risk of elevated PP (multiple testing corrected, P_{val}<0.05/3)($\beta_{T2D\rightarrow PP}$ [SE]=0.37[0.021]) and vice versa ($OR_{PP\rightarrow T2D}$[95%CI]=1.02[1.01-1.04]). MR suggested a causal effect driven by T2D on PP, but highlighted moderate heterogeneity (due to the heterogeneity of T2D itself). Partitioning genetic variants into clusters of pathogenetic processes highlighted two remarkable groups: Cluster1 was associated with an inverse T2D/BP relationship and Cluster2 with risk of vascular dysfunction in cardiovascular disease, related to features of Metabolic syndrome, including central obesity, liver dysfunction, insulin resistance and shorter stature. Using cluster-partitioned PGSs, we highlighted a reciprocal prediction of risk of T2D and elevated PP within Cluster1 and Cluster2 ($\beta_{T2D\rightarrow PP\ Cluster1}$[SE]=-0.57 [0.020] ; $\beta_{T2D\rightarrow PP\ Cluster2}$[SE]=0.67[0.020],all *P-values* < 1.00×10^{-10}). MR using variants from Cluster2 revealed a deleterious circle of causality between DBP, T2D, and SBP, each increasing the risk of comorbidity ($OR_{IVW\ DBP\rightarrow PP\ through\ Cluster2}$[95% CI] = 1.04 [1.02-1.06], *P-value*=1.86×10^{-5} ; $\beta_{IVW\ T2D\rightarrow SBP\ through\ Cluster2}$[SE]=1.987 [0.278], *P*-value = 7.25×10^{-25}).

Partitioning SNPs by pathogenetic process enabled mechanistic insights into causal effect of T2D leading to altered BP related to symptoms of Metabolic syndrome.

Francesc PUIG-CASTELLVÍ

Drug x Microbiome x Metabolome Interactions in a Population with Type 2 Diabetes

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Individuals with type 2 diabetes (T2D) frequently experience additional chronic conditions such as hypertension, hyperlipidemia, and obesity[1]. These comorbidities significantly influence the treatment and management of T2D because the use of multiple medications to address both the primary disease and these concurrent health issues can exacerbate dysbiosis in the gut microbiome and have repercussions on their circulating metabolome[2].

The objective of this study was to tease out the drug x microbiome x metabolome interactions in the METACARDIS type 2 diabetes population (n = 2,194) using ultra-performance liquid chromatography coupled to high resolution mass spectrometry (UHPLC-HRMS) and metagenomics[3], to 1) identify the drugs with the largest impact in the host metabolome and 2) evaluate the microbiome contribution to the metabolomic signature associated with medication.

We found out that metformin is one of the T2D drugs producing the largest metabolic alterations in the serum, with more than 300 metabolites impacted by metformin intake. At the gut microbiome level, metformin is associated with *Lachnoclostridium*, *E.coli, Bifidobacterium* and *Firmicutes*. The functional roles more altered by metformin intake were linked to sugar transport, biosynthesis and degradation.

Altogether, we demonstrated that the effects of drug treatment are partly dependent on patients' poly-pharmacy and on their gut microbiome. These insights can lead to improved treatment strategies, better management of comorbidities, and a deeper understanding of the mechanisms underlying T2D and its treatment.

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Omolara TIJANI

Impact of glucocorticoids on islet function - Role of *SRD5A1* as a modulator of glucocorticoids bioavailability

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Background and objectives: The emergence of diabetes as a side effect of therapeutic drugs, particularly glucocorticoids (GC), is a matter of substantial concern. GC are widely prescribed for their immunomodulatory and anti-inflammatory properties. Approximately 1% of the general population is on chronic GC therapy, and nearly half develop glucocorticoid-induced diabetes (GCID). The local bioavailability of GC, which significantly influences their metabolic impact, is closely associated with their degradation. The enzyme *SRD5A1* plays a pivotal role in reducing GC bioavailability by metabolizing them into the metabolite 5a-THF. Human subjects on *SRD5A1* inhibitor, as well as rodent models with *SRD5A1* knockout, exhibit impaired insulin sensitivity and elevated risk of pre-diabetes. Consequently, we hypothesize that enhancing *SRD5A1* enzymatic activity could potentially ameliorate the detrimental effects of GC on pancreatic islets and offer a preventative measure against GCID.

Methodology: To examine the impact of GC, on glucose-stimulated insulin secretion (GSIS), we conducted dynamic perifusion on islets from 5 donors pancreata treated with prednisolone (PRED) 250 nM, 500 nM, 1 µM for 24h. For SRD5A1 expression, we utilized RNAscope analysis and Western blot on islets from 8 human pancreata. To assess SRD5A1's basal activity, islets were treated with 500 nM hydrocortisone for 24h, and GC metabolites were quantified in the islet supernatant through HPLC-MS/MS (n = 4). We also performed *SRD5A1* mRNA transfection in human islets (n = 2) to overexpress *SRD5A1* protein.

Results: Our findings indicate: 1. A PRED concentration of 250 nM significantly reduces GSIS. 2. *SRD5A1* is the predominant GC degrading gene expressed in the human islets, its localization is higher in the *β*-cells of the lean phenotype, while in the obese and T2D phenotype, it is more strongly expressed in exocrine cells. 3. HPLCMS/MS analysis revealed limited basal activity for *SRD5A1* in GC degradation. 4. Transfection of *SRD5A1* mRNA into human islets led to an increase in protein expression.

Conclusion: In a novel vitro, we established that 250 nM PRED (average peak blood level after a 5 mg oral PRED dose) effectively reduces GSIS. *SRD5A1*, the primary enzyme for GC degradation in human islets, exhibits low basal endogenous activity. Ongoing research is focused on confirming the consequences of *SRD5A1* overexpression on GC metabolism and its influence on pancreatic hormone secretion.

Ninon VERY

O-GlcNAcylation controls pro-fibrotic transcriptional regulatory signaling in hepatic stellate cells

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[1]*U1011 – EGID*
[2]*U1286 – INFINITE*
[3]*UAR 3290 – MSAP*
[4]*UMR7178 – IPHC*

OBJECTIVES: Liver fibrosis is characterized by excessive accumulation of extracellular matrix (ECM) resulting from persistent wound healing in chronic liver injuries leading to liver architecture and function disruption. Current therapeutic options for liver fibrosis are limited, necessitating a deeper understanding of molecular mechanisms, especially those that control the activation of hepatic stellate cells (HSCs). Upon liver injury, “quiescent” HSCs (Q-HSCs) transform into a highly contractile and ECM-producing myofibroblastic state (MF-HSC). Although this process is triggered by deep metabolic and transcriptional reprogramming, functional links between these events are not yet understood. This metabolic shift is reminiscent of changes observed in cancer cells (Warburg effect), where it is accompanied by increased protein O-GlcNAcylation, a nutritional sensor post-translational modification (PTM) that regulates various proteins including transcription factors (TFs). To date, only sparse and conflicting results on the role of O-GlcNAcylation in MF-HSCs have been reported. We hypothesized that O-GlcNAcylation may connect the metabolic shift with the transcriptomic reprogramming characterizing and underlying MF-HSC activities.

METHODS: We employed in vitro (human LX-2 HSC cell-line), ex vivo (mouse and human primary HSCs, murine Precision-Cut Liver Slices (PCLSs)) and in vivo (CCl4 fibrotic murine livers) experimental models to access O-GlcNAcylation levels between Q-HSCs and MF-HSCs as well as healthy and fibrotic livers. Specific inhibition or knockdown strategies targeting O-GlcNAc Transferase (OGT) combined to a multi-omics approach (proteomic, epigenomic and transcriptomic data) were used to determine the impact of O-GlcNAcylation on MF-HSC pro-fibrotic activities and transcriptional program.

RESULTS: We found that O-GlcNAcylation is increased during HSC myofibroblastic activation and is required for their pro-fibrotic activities. Mechanistically, O-GlcNAcylation controls the transcriptional program of MF-HSCs by targeting key transcriptional regulators (TRs) including Basonuclin 2 (BNC2) and TEA domain transcription factor 4 (TEAD4) fibrogenic TFs.

CONCLUSION: This study provides new insights into the molecular mechanisms underlying HSC activation and defines inhibition of O-GlcNAcylation as a potential therapeutic strategy to treat liver fibrosis.

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