Abstracts

8th Santorini Conference Systems Medicine and Personalised Health and Therapy
Santorini, Greece, 3–5 October 2016

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Monday 3 October, 2016

Session I – FROM SYSTEMS BIOLOGY TO SYSTEMS MEDICINE

From the 1st to the 8th Colloquium

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Twenty years ago, Gerard SIEST visited for the first time Santorini. I can’t say that I remember much or that it enchanted him. I remember a terrifying in my eyes, uphill dirt road from the port to the main part of the island, a black, very windy beach but, also, a magical view to the caldera and a unique, breathless, extremely serene and plenty of dreams smile on Gérard’s face while loosing his gaze in the horizon. The island had bewitched him! It became his small paradise…

And in 2002, the story begins…

The “1st Santorini Conference “From Genetic Variations to Risk Prediction and Pharmacogenomics” was organised on 25-28 September 2002!

In some outstanding cases, inspiration confirms the talent and resulting work speaks for itself. And Gerard SIEST was one of those cases, whose ideas have taken the “Santorini Conferences” by storm.

Throughout the 14 years, 8 “Santorini Conferences” welcome to a world of passion of science with specific Personalised Medicine sessions on Genetics and Pharmacogenomics of risks and chronic diseases, each time, more than 150 registered participants coming from more than 30 different countries, 50 invited speakers, 20 selected oral presentations and 50 posters.

From innovative projects, such as the creation of the European Society of Pharmacogenomics and Personalised Therapy (ESPT) in 2010 and upcoming research to the quintessence of personalised medicine with hidden and famous researchers, the “Santorini Conferences” had many faces and like a chameleon had the capacity to enchant academic and industry research in many ways.

Over the years, the “Santorini Conferences” became one of the most important conferences on genetic predisposition to health, disease, response to drugs and environment in harmony with the island creative spirit attracting scientists from all over the planet. None can dispute the unique view of Gerard!

After 14 years, we are here again to follow the path along the caldera of the 8th Santorini Conference “Systems medicine and Personalised Health and Therapy” (3-5 October 2016, http://santoriniconference.org/) that Gerard had already constructed before passing away. The time has come to honour the memory of Gerard and our scientific guests and sponsors!

You will never get bored on this conference, that’s for sure!

Just continue reading…

Mechanistic bioprofiles and pharmacophenomics for precision medicine of heart failure

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Heart failure is a major and growing public health problem, with high rates of morbidity and mortality. The complexity of standard medical treatment for heart failure is growing, and such therapy typically involves five or more different medications. Given these pressures, there is increasing interest in harnessing cardiovascular biomarkers for clinical application in order to more effectively guide diagnosis, risk stratification, and therapy. By tailoring medications for specific individuals, it may be possible to realize an era of personalized medicine for heart failure treatment in which therapy is optimized and costs are controlled.

Personalized medicine is the practice of obtaining non-obvious information about an individual, such as that contained in biomarkers, for the purpose of guiding therapeutic decisions tailored to that individual. In oncology, biomarker testing is used to identify treatments for highly specific molecular targets in order to match effective therapy to specific populations and thereby improve patient tolerance to therapies that have toxicity profiles that would be unacceptable in an unselected population. The clinical utility of biomarkers in cardiology has been less clear, because usual practice groups several pathways leading to heart failure and selection of therapies. Reasons for using biomarkers to guide therapy include challenges in optimizing therapy and utility for risk stratification and prognosis.

Such tailored therapy is becoming increasingly common in the field of oncology, but the direct mechanistic coupling of biological processes and treatments achieved in the realm of cancer treatment remains elusive in heart failure. The concept of the Heart ‘omics’ in AGEing (HOMAGE) project, an EU FP7 program we are coordinating at Inserm, University of Lorraine, Nancy, France, www.homage-hf.eu) is to identify ‘omics’-based biomarkers that can detect pathological processes predictive of the development of heart failure to allow early mechanistically driven therapeutic interventions for preventing heart failure.

Recent clinical trials and meta-analyses of biomarkers in heart failure have produced conflicting evidence. The background and rationale for biomarker testing in heart failure is creating opportunities faced by challenges related to building trial based evidence that may facilitate the regulatory pathway.
Session II – SYSTEMS PHARMACOGENOMICS AND MECHANISMS OF DRUG ACTION

Metabolomics & Its Role in Precision Medicine – From Patient to Drug

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Advances in gene sequencing have yielded massive quantities of data and resulted in many important insights. However, a staggeringly complex picture has also emerged, and data production is clearly outpacing the ability to discern actionable signals from it. Further, many traits are multifaceted, with influences distributed across the genome, microbiota and environment. Sifting through this data and complexity to identify disease and response biomarkers and signals that precisely define drug action is essential for advancing precision medicine. Metabolomics provides a snapshot of the current state of the phenotype and accounts for these diverse influences, making it instrumental in precision medicine. This phenotypic snapshot, and the metabolic ontologies embedded within it, has been a key for triaging large volumes of gene sequencing data to unravel complex traits and detect meaningful signals within.

Major technological innovations enable metabolomics to deliver signatures of disease and response at the individual level and perform as an integral tool for assessing drug mechanism. By offering a comprehensive, real-time assessment of the phenotype, and defining meaningful signals within the genome, metabolomics is expanding the boundaries of precision medicine and systems pharmacogenomics.

Prediction of combination therapy based on perturbation modeling of the immune system signaling network in patients with Multiple Sclerosis

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Modeling signaling pathways provides insights about the cell response to environmental changes and drug effects. Development of combination therapies is hampered because difficulties in predicting at the molecular level the best combinations achieving a given biological effect and to predict how it would translate at the clinical level for each subgroup of patients, considering disease heterogeneity. Also, because the combinatorial nature of such studies imply an enormous number of experiments and associated costs, preventing an in depth analysis for all the alternatives. Finally, because the difficulties of running clinical trials with more than one drug, which would require large trials to reach power, and the lack of incentives of pharmaceutical industry for exploring this approaches, the field of combination therapy has not deployed its full potential.

We have developed (CombiMS EU project) a systems medicine approach to (i) characterize the signaling pathways that mediate Multiple Sclerosis (MS) in primary immune cells obtained from patients and controls and (ii) predict new combination therapies based in logic networks simulations. Towards this end, we assembled a literature-derived prior signaling network of the key pathways involved in immune signaling in MS. We collected measurements of the phosphorylation of key proteins upon perturbation with ligands and drugs across a set of 150 patients and 50 controls. These phospho-measurements were used to train the PKN, thereby identifying the single active network that best fitted the data for each patient. Also, we identified specific networks for patient subgroups and drugs. We hypothesized that the defective interactions should be those with a signaling value more distant between healthy and treated patients than between healthy and MS patients. Subsequently, we developed an approach for predicting combination therapy based on network topology: using a simple graph search, we identified the stimulus used in our in-vitro assays that activated interactions found to be defective with each drug. Finally, we mapped disease phenotypes and MS therapies with each pathway signature and enriched the analysis by genotyping 110 SNPs associated with MS. By identifying kinases and pathways associated with a given phenotype, we searched for combinations of drugs tested in the in vitro assays or identified in DrugBank in order to target different combinations of pathways in order to approach the healthy state profile. We validated these findings by flow cytometry, identifying the cell subtypes contributing to each signaling signature, as well as validated some combination therapies in the animal model of MS. The pipeline showed here can be used for developing combination therapies for other complex diseases.

Genomic precision to be achieved in the use of VEGF-inhibitors in cancer patients

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The field of pharmacogenomics is focused on the characterization of genetic factors contributing to the response of patients to pharmacological interventions. Drug response and toxicity are complex traits; therefore the effects are likely due to multiple genes. The investigation of
the genetic basis of drug response has evolved from a focus on single genes to relevant pathways to the entire genome. Large clinical trials enriched with genome-wide association studies provide an unprecedented opportunity for a comprehensive and unbiased assessment of the heritable factors associated with drug response. In oncology, germline genomics is still relatively unexplored, particularly in reference to biomarkers of patient survival. This is particularly relevant for angiogenesis inhibitors. Despite the survival advantage conferred by anti-angiogenic therapy, this treatment strategy still faces a number of barriers, including an association with significant toxic side effects and the high cost of bevacizumab therapy. The failure to identify clinically useful biomarkers that can consistently predict clinical efficacy of this class of agents has also proved to be a significant hurdle. Even in studies where bevacizumab has provided minimal or no survival advantage, groups of patients with long progression-free survival could be identified. Genomic studies on bevacizumab are prototypical to address these barriers.

After approval in 2004 as the first anti-VEGF drug, the expanded use of bevacizumab beyond metastatic colorectal cancers included selected patients with advanced lung, renal cell, ovarian, breast, and cervical cancers, and patients with glioblastoma. Now the drug is used mostly in combination with chemotherapy by many patients in the US and worldwide. Unfortunately, still no biomarkers exist to target the appropriate patients for bevacizumab therapy or to offer alternatives that allow those patients unlikely to benefit from bevacizumab to avoid the associated toxicities and high financial costs. Bevacizumab treatment is usually accompanied by elevation in blood pressure, kidney toxicity, and many other side effects. In rare instances, more severe side effects of bleeding, heart attacks, heart failure, or blood clots are reported. These events have a mechanistic basis and are dose dependent.

This talk will be focused on the discussion of studies using germline genomics in cancer to discover the genetic determinants of efficacy and toxicity of VEGF inhibitors.

Session III – CELLULAR TARGETS IN ONCO-HEMATOLOGY

Stem cell transplantation and pharmacogenomics

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Hematopoietic Stem Cell Transplantation (HSCT) is an established treatment option for patients with malignancies and non malignancies. There is a perpetual rise in the number of allogeneic HSCTs in children suffering from leukemia and primary immunodeficiencies. HSCT related complications such as sinusoidal obstruction syndrome (SOS), acute graft versus host disease (aGvHD), infections, and organ failure are the major causes of mortality and morbidity in addition to disease related causes. Stratification of patients using a priori pharmacogenetic, demographic and clinical information might aid in personalized management, and thus optimize treatment, minimizing adverse events. For example, using genetic data for personalized dosing, might help to achieve optimal outcomes, particularly when patients are receiving a drug such as busulfan (BU) which has a narrow therapeutic window and exhibits wide inter-individual variability in its pharmacokinetics.

In this presentation, as an example, the results of a multicentric study that investigated the association of genetic variations in genes encoding glutathione S transferase enzymes with BU pharmacokinetics and clinical outcomes in a pediatric HSCT setting will be discussed. The results demonstrated that although all the patients had BU dose adjustment after therapeutic drug monitoring to achieve a target steady state concentration, the initial unadjusted dose drug exposures still suggested being responsible for certain adverse events, especially in patients with slow metabolizer enzyme capacity. This has been demonstrated by higher incidences of treatment related toxicity (62.5%) in patients requiring dose reduction compared to those who required dose increments (27%). There was about 23% increase in BU clearance in patients carrying glutathione S transferase alpha (GSTA1) haplotype (*A2*A2) producing a rapid metabolizing enzyme. There were higher incidences of aGvHD (65%) in patients carrying two slow metabolizer haplotypes of GSTA1 (*B*B) compared to those carrying rapid metabolizing enzyme (15%). Furthermore, there was an increase of aGvHD incidences in patients carrying both non functional GSTP1 (*B*B) and GSTA1 (*B*B) haplotypes in homozygous condition (75%).

Observations from this study reveals that, there is the possibility of optimizing BU first dose prior to therapeutic drug monitoring with the help of GSTA1 genetic information, in addition to age and/or weight to minimize variability in BU exposure by reducing doses in GSTA1 *B*B carriers from the start. In addition the option for patients to receive alternate conditioning or prophylactic measures is also possible based on genetic information. This will aid in decreasing consequences like aGvHD, and treatment related toxicity due to inaccurate first and subsequent dosing.

Tumor immunity and immunotherapy: past, present, future

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After a century of skepticism, doubts and controversies, it is now well established that the immune system has the capabilities to control tumor growth, offering the possibility to exploit this property to design diverse types of immunotherapy. Tumor immunity could be seen...
as a delicate balance between anti-tumor mechanisms and multiple subterfuges used by the tumor to escape immune attacks. Thus, tilting this balance in the good direction is the main principle of diverse strategies, such as vaccines, cell therapy, or reshaping the microenvironment. Therapeutic vaccines have been developed along the last 25 years without the expected success. The main causes of this failure will be described, as well as the approaches currently explored for improvement. T cell therapy was pioneered with the adoptive transfer of tumor-infiltrating lymphocytes, paving the way of molecular engineering approaches such as tumor-specific T cell receptor transgenic and chimeric antigen receptor T cells. Impressive results have recently been achieved in the fields of leukemia and lymphoma mainly, with the hope for future similar benefit for patients with solid tumors. The therapeutic potential of immunotherapy may be reinforced by counteracting some of the main mechanisms making the tumor bed hostile to effector cells of the immune system. Main efforts are ongoing to inhibit local and systemic immunosupression mediated by soluble factors or regulatory cells such as myeloid derived suppressor cells. The spectacular results obtained in the last years with immune checkpoint inhibitors, used as single molecules or in combination with other immunotherapeutic modalities, will also be summarized. Finally, how the synergy between immunotherapy and other treatment modalities (e.g., irradiation, chemotherapy, targeted therapy, oncolytic virus) is currently explored will be introduced.

Multiple myeloma targets

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Multiple myeloma is characterized by the proliferation of clonal plasma cells in the bone marrow and the secretion of monoclonal immunoglobulins. This second most common hematologic malignancy was incurable with a median survival of 2 to 3 years until the 1990s. Together with autologous stem cell transplantation and advances in supportive care, the use of two novel classes of active agents (such as proteasome inhibitors and immunomodulatory drugs) has increased response rates and survival substantially in the past several years. The understanding of the biology of myeloma increased by description of deregulation of the RAS/MAPK, NFkB pathway, apoptotic response, and additional oncogenic events, including activation of oncogenes (K-Ras, N-Ras, FAM46C, MYC, BRAF), loss of function of p53, epigenetic changes and genomic instability. Genomic and epigenomic studies have started to unravel the mutational landscape, clonal heterogeneity, and patterns of clonal evolution in multiple myeloma. These finding are ongoing to help personalize treatment and identify additional novel molecular targets of therapy: second- or third-generation proteasome inhibitors / immunomodulatory drugs, histone deacetylase inhibitors, and monoclonal antibodies. In 2015, four new drugs were approved by the US Food and Drug Administration. Cereblon, histone deacetylase, SLAMF7 and CD38 are the main targets of therapeutic interest currently explored in multiple myeloma.

Leukemia Pharmacogenomics

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Background: Treatment of childhood acute lymphoblastic leukemia (ALL), a malignant disorder of lymphoid progenitor cells, is a constant struggle to choose the perfect dosing regimen that would ensure maximum benefit and minimum toxicity. Although advances in the treatment of ALL have greatly improved treatment success rates to exceed 80% in favorable settings, this masks the fact that treatment-related toxicity can be life-threatening and is a main cause of treatment interruption or cessation.

Objective: Given the marked heterogeneity in responses to ALL treatment, a better understanding of the molecular basis for inter-individual differences in drug effects has the potential to significantly improve the efficacy and reduce toxicity of chemotherapy. A comprehensive understanding of the contribution of genetic signature to therapeutic responses in childhood ALL is required so that these variants can be used to personalize treatment. The objective of this work was to apply exome analysis to uncover variations associated with treatment-related complications in ALL.

Design: We performed whole-exome sequencing (WES) in Quebec childhood ALL (QcALL) cohort from CHU Sainte-Justine, Montreal providing a catalogue of variations in childhood ALL genomes. Using integrative computational strategies on the available dataset, we performed exome-wide association studies with ALL treatment complications. The analysis focused on exonic common variants with minor allele frequency ≥ 5% that are further filtered on the functionality and are thus predicted to affect protein structure and function.

Results: The results obtained by an association analysis of WES data with the peripheral neuropathy, a dose-limiting toxicity of vincristine (VCR) and with asparaginase-related complications, including thrombosis, pancreatitis and allergies, are presented. Following correction for multiple testing several top-ranking SNPs were identified. Association signals were subsequently confirmed by genotyping and through replication studies.

Conclusions: The results provide additional insight into the novel pharmacogenetics markers of adverse drug events in ALL obtained through new generation sequencing.
References:

Acknowledgements: This work was supported by Leukemia and Lymphoma Society of Canada, Canadian Institute of Health Research, Foundation Cole and Foundation Charles Bruneau.

Session IV – GENOMICS AND PROTEOMICS OF ALZHEIMER’S DISEASE

African-American TOMM40 523-APOE haplotypes are admixture of West African and Caucasian alleles, not present in other ethnic groups

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Background: African Americans have an increased age-related prevalence of AD yet have a lower APOEe4 allele frequency compared to Caucasians. In Caucasians, greater than 98% of APOEe4 alleles are connected to the Long polyT repeat [L] of TOMM40 [Translocase of Outer Mitochondria Membrane] within the LD region containing both genes. Thus APOE4 and TOMM40-L carriage are both related to age of onset distributions in Caucasians. African-Americans have a higher allele frequency of the TOMM40-L allele, suggesting the possibility of a haplotype found in African-Americans and not in Caucasians.

Methods: We have compared the APOE and TOMM40’523 phased haplotype frequencies of a 9.5 kb TOMM40/APOE genomic region in West African, Caucasian and African-American cohorts.

Results: African-American haplotype frequency scans of polyT lengths connected in phase with either APOE4 or APOE3 differ from both West Africans and Caucasians and represent admixture of several distinct West African and Caucasian haplotypes. A new West African TOMM40’523 haplotype, with APOE4 connected to a Short allele, is observed in African-Americans but not Caucasians, accounting for an excess of APOE3 alleles compared to APOEe4. The polyT length of the S in this 4-S haplotype in S-15, while S-16 is the predominant polyT length in Caucasians when attached to either APOEe3 or APOEe2.

Conclusion: Phase-sequencing allowed the demonstration of the high proportion of S15 alleles connected to APOEe4 in the African-Americans that are rare in Caucasians. Before the demonstration of APOE4-S haplotypes, the use of APOE4 alone underestimated the L effect in African-American populations. TOMM40 genotypes are informative for >97% of the Caucasian population, and APOE4 containing genotypes were informative for about 30% of the APOE4 carriers. Each of the three APOE3/3 genotypes [S-S, S-VL, and VL-VL] genotypes are now informative for Caucasians analyses, but a new haplotype of APOE3-L will be necessary for accurate age of onset distributions in African-Americans. Data will be presented to support admixture of the West African population and Caucasians to explain the introduction of APOE3-L into the African-American population. These data have therapeutic implications for age of onset risk algorithm estimates and the design of a prevention trial for African-Americans or other mixed ethnic populations.

Functional Analysis of the APOE/TOMM40 Locus Implicates a Complex Regulatory Structure

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Genetic variation within the apolipoprotein E (APOE) locus is associated with late-onset Alzheimer’s disease risk and quantitative traits as well as apoE expression in multiple tissues. To explore whether APOE locus cis-regulatory elements might contribute to regional gene regulation we produced regulatory region reporter constructs containing haplotypes of: 1) APOE locus promoters for APOE, APOCI and TOMM40, 2) potential enhancers; TOMM40 intervening sequence (IVS)2-4, TOMM40 IVS6 poly-T, as well as 3) previously described enhancers; APOE exon 4, multi-enhancer 1 (ME1), or brain control region (BCR). These regulatory region cis-elements were evaluated for their effects on luciferase activity in multiple human cell lines. Results of this investigation demonstrate that in SHSY5Y cells the APOE promoter is significantly influenced by TOMM40 IVS2-4 and ME1 according to haplotype. In addition, in SHSY5Y cells the TOMM40 promoter is significantly influenced by APOE exon 4, TOMM40 IVS6 poly-T, ME1 and BCR according to haplotype. The TOMM40 promoter is significantly influenced by in APOE exon 4, TOMM40 IVS2-4, ME1 and BCR in HepG2 cells according to haplotype. Whereas the APOE promoter is influenced by APOE exon 4 haplotype
in HepG2 cells. The main novel findings are that multiple APOE locus cis-elements influence both APOE and TOMM40 promoter activity according to haplotype and cell type, suggesting that a complex regulatory structure modulates regional gene expression. These results have important implications for Alzheimer’s disease therapeutic strategies that focus on targeting APOE expression.

Interactomics, identification of small molecules and prediction of putative disease modules for Alzheimer’s disease and other neurodegenerative diseases

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Protein misfolding diseases such as Alzheimer’s disease (AD), Parkinson’s disease (PD), and Huntington’s disease (HD) are characterized by the accumulation of insoluble protein aggregates in patient brains. An increasing body of evidence indicates that soluble, seeding-competent amyloid oligomers rather than mature fibrils or amyloid plaques are the major toxic species in amyloid diseases. The exact nature of pathogenic aggregates and their mechanisms of toxicity in cells, however, are largely unclear.

We use network biology and various OMICs approaches to identify proteins and small molecules that can influence abnormal protein misfolding and aggregation in neurodegenerative disease model systems. For example, we recently generated a large interactome network connecting multiple proteins involved in different neurodegenerative diseases (NDs) that was termed NeuroNet. It links a large number of known neurodegenerative disease-causing proteins (NDCPs) such as huntingtin, α-synuclein or TDP-43 to novel partners and thereby facilitates the discovery of proteins that potentially influence abnormal protein aggregation in various models of neurodegenerative disorders such as AD or HD. The network facilitated the prediction of highly connected “neurodegenerative disease modules” that contain known NDCPs from different NDs, therapeutic targets and regulators of abnormal protein aggregation. These disease modules highlighted proteins that are abnormally aggregated in brains of AD patients. Finally, we used a chemical genomics approach to identify small molecules that perturb protein misfolding and aggregation in vitro and in vivo. This strategy allowed the discovery of chemical compounds such as DO1 that directly targets and dissociates seeding-competent, β-sheet-rich fibrillar Aβ oligomers and delays the onset of seed-mediated Aβ polymerization in vitro. Furthermore, we show that DO1 treatment of transgenic 5xFAD AD transgenic mice reduces the size of fibrillar Aβ plaques and concomitantly improves memory and social impairment. Our studies suggest that interactome maps are valuable resources that enable the elucidation of common disease mechanisms. Furthermore, through the application of OMICs strategies and computational tools novel target proteins as well as potential therapeutic small molecules can be identified.

The case of genetic determination of leukocyte telomere length in childhood

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Background: Approximately 30 million people currently suffer from late-onset Alzheimer’s disease (LOAD) worldwide. Twin studies demonstrated that 60 to 80% of LOAD is genetically determined, 20% of which remaining unassigned.

Objective: The objective was to identify additional SNP candidates that are associated with MCI.

Design: This case-control study included 118 cognitively healthy controls, 52 patients with mild cognitive impairment (MCI; the pre-stage of LOAD) and 71 LOAD patients. The participants were genotyped for the genetic LOAD marker apolipoprotein E4 (APOE4) and the single-nucleotide polymorphism rs4925 in glutathione S-transferase omega-1 (GSTO1). Genotyping results were analyzed by logistic regression using a dose-dependent model.

Results: Additive logistic regression showed a novel, statistically significant association of the major allele GSTO1*C with MCI (OR1.9; p = 0.032). However, identification of significant SNP-disease relations required well-defined study groups. When classifying participants solely by the short Mini Mental State examination (MMSE), the associations of GSTO1*C and the reference marker APOE4 with MCI were cancelled. Moreover, even identifying only the control group by MMSE nullified a statistically significant association (OR1.8; p = 0.045) between GSTO1*C and LOAD. In contrast, these statistical relations were retained when the detailed Consortium to Establish a Registry for Alzheimer’s Disease (CERAD-Plus) test battery was used.

Conclusions: Hence, besides proposing rs4925 as a genetic marker for cognitive impairment, this study also emphasized the importance of carefully characterized controls in addition to well-diagnosed patients in case-control studies.

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Session V – OMICS STUDIES OF HUMAN PHENOTYPES AND ENVIRONMENT

Insights to coronary artery disease genetics through UK Biobank

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Coronary artery disease (CAD) is the commonest cause of death in the world. Genome-wide association studies (GWAS) have identified so far 56 robust associations ($p<5 \times 10^{-8}$) for CAD explaining ~15% of the heritability of the disease. Most of these signals are driven by common variants with low-frequency associations found in only a handful of genes (LPA, PCSK9, SVEP1 and ANGPTL4). However, such variants seem to contribute minimally to the genetic architecture of CAD.

Around a third of CAD loci show association with a known or putative cardiovascular risk factor, in particular blood pressure and lipid traits. Furthermore, several loci also show association with other diseases: for example, the CAD-associated variants in the chromosome 9p21 locus also associate with risk of stroke, abdominal aortic and intracranial aneurysms. In an ongoing comprehensive analysis of variants associated with other diseases and traits we identified six new loci associated with risk of CAD providing important insights on genetic mechanisms shared by different diseases.

UK Biobank was established to improve understanding of the causes of common diseases including CAD (www.ukbiobank.ac.uk/) and completed the recruitment of 502,713 (94% of self-reported European ancestry) individuals aged 40-69 from around Great Britain between 2005 and 2010. In addition to self-reported disease outcomes as well as extensive health and life-style questionnaire data, participants are being tracked through their NHS records and national registries (including cause of deaths, hospitalisations and primary care records). Based on these data, we identified 10,801 CAD cases with available genetic data (July 2015 release; data imputed to the 1000 Genomes panel) and undertook an association analysis combining all previous studies. Based on initial results, there are several new CAD risk loci which are however once again driven by common variants.

Update on the exposome in Europe and in the states

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Background and aim: The exposome concept has been proposed by Chris Wild in 2005 and consists in the integration of all exposures over the life time. It has two types of components: a space component, ie the integration of different exposures including diet, environmental pollutants, physical and biological stress as well as psychosocial stress; the second component is time, as it aims at considering exposures from conception (or even before) to death. This extremely ambitious concept is expected to complement the genome approaches and to account for environmentally driven diseases. The different exposome projects in Europe and in the United States will be briefly discussed.

Results and conclusions: There are several EU FP7 projects, which focus on the exposome, Exposomics, Helix and Heals. Exposomics aims at developing methodologies to primarily assess the effects of air pollution and water contaminants. Helix focuses on early life exposure. Heals develops a conceptual framework integrating health studies including a panel of omics approaches, exposure and PBTK modelling and toxicological approaches. The ambition of Heals is to provide a model faithfully describing the different scales, from cell to population. Heals also includes the launching of a twin study in several EU countries.

The NIH has funded a large exposome program called Hercules. It includes a robust facility providing analytical support to large scale epidemiological studies across the US.

Non alcoholic fatty liver disease (NAFLD): interaction between phenotype and environment

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Non alcoholic fatty liver disease (NAFLD) is one of the biggest public health challenges facing modern times as it affects up to 25% of the European population. The incidence and prevalence of NAFLD are rapidly growing and more attention is given to this disease. NAFLD is diagnosed when the amount of hepatic triglycerides is greater than 5.6%. It includes a wide spectrum of liver diseases like non alcoholic steatohepatitis (NASH), fibrosis and cirrhosis and it is currently the most common cause of chronic liver disease.
NAFLD is often associated with hepatic and systemic inflammation, insulin resistance (IR) and obesity. However, also non obese subjects, mainly males, might have NAFLD especially if they develop abdominal obesity. NAFLD is a metabolic disease since lipotoxicity and IR, in particular in the adipose tissue, are among the main risk factors for the development and progression of this liver disease that can result also in cirrhosis and hepatocellular carcinoma (HCC), leading to liver transplantation (LT) and death. Currently, there is no approved treatment for NASH, and low-calorie diet and physical exercise is generally recommended to reduce body fat and improve IR.

The mechanisms that lead to NAFLD are still not completely clear. The “first hit” is hepatic steatosis, due to high caloric intake and sedentariness. On the other hand the “second hit” that might lead to NASH and HCC includes derangement in lipid metabolism, lipotoxicity, but also some environmental factors, like changes in gut microbiota and bacterial translocation, and exposure to pollutants like phthalates and bisphenol A that can act as endocrine disruptors. Although some genes have been associated with predisposition to NAFLD and NASH, like PNPLA3, GCKR, TM6SF2, TCF7L2, APOC3, DGAT, f, they are somehow not associated with increased IR. Indeed, it is the presence of IR that makes the subjects more at risk of dangerous lipid profile and progression of liver disease and related comorbidities, as type 2 diabetes and cardiovascular disease.

The new “omics” techniques including genomics, transcriptomics, epigenomics, metabolomics, exposomics and foodomics are currently used in clinical trials to identify mechanisms and risk factors and hopefully in the future they will be used to personalize therapy and cure this metabolic disease.

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The human plasma-metabolome: reference values in 800 healthy volunteers; impact of cholesterol, gender and age

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Metabolomics is increasingly used to identify new biomarkers of numerous diseases, but normal values of plasma metabolites remains poorly defined. The aim of the present study was to define the “normal” metabolome in healthy volunteers.

895 French healthy volunteers from 18 to 86 years old, equally distributed between men and women, free of any medication, and considered healthy based on medical history, clinical examination and standard biology were included. 185 targeted plasma metabolites including amino acids, biogenic amines, acylcarnitines, phosphatidylcholines, sphingomyelins and hexoses were quantified by tandem mass spectrometry using Biocrates Absolute IDQ p180 kit. Principal component analysis was used to identify the main components of metabolome variability.

We established plasma metabolite references dataset for these 185 metabolites. Total blood cholesterol, gender and age were identified as the main components explaining the variability of the studied human metabolome. High levels of total blood cholesterol were associated with higher sphingomyelin and phosphatidylcholine plasma concentrations. Gender associated differences highlighted that sphingomyelin levels were lower in men than in women, contrary to lysophosphatidylcholine levels (higher in men than in women). Finally, elderly healthy volunteers had higher sphingomyelin and phosphatidylcholine plasma levels than young healthy volunteers.

Reference: human metabolome values were established on a large and well-defined healthy volunteer’s population. This study provides an essential baseline to define the normal metabolome profile and the main sources of variation.

Session VI – NUTRITION AND METABOLIC HEALTH

Genetics of Nutrition and of metabolic diseases

Ph. Froguel

Imperial College London and CNRS-Pasteur Institute and Lille University.

Obesity is a genetic trait with 70% heritability. In addition, mutations in gene mainly part of the regulation of food intake have been found to cause monogenic obesity. Furthermore, GWAS have identified more than 100 loci increasing BMI. We have evidence that different parts of the
Gene-nutrient interaction data stemmed from nutritional interventions. The paradigm of the MAST4HEALTH EU program

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Nonalcoholic fatty liver disease (NAFLD) includes a spectrum of liver injury affecting approximately 50% of diabetics and 76% of obese patients. Since current NAFLD treatments are limited, there is the emergence in exploring novel means of managing NAFLD. We aimed on exploring non-pharmacologic means for managing the disease and in particular through dietary substances or bioactive phytochemicals in fruits, vegetables, and plants or their products. An intervention trial with Corinthian raisins was applied in 55 patients with NAFLD. The participants were randomly assigned into two isocaloric dietary treatments for 24 weeks (a) nutritional counselling within the Mediterranean dietary pattern (N=27) (b) nutritional counselling according to the Mediterranean pattern with black currants included (two fruit servings, 36 g/day) (N=28). Anthropometric, blood pressure, assessment of MedDietScore, NAFLD Fibrosis Score, imaging control (hepatic ultrasound, ShearWave Elastography) and biochemical tests were conducted pre- and post-intervention. A total of 50 patients completed the trial. Within the intervention arm a decrease of fibrosis stage, CRP, glucose, leptin and IL-6 levels remained significant. Furthermore, patients with at least one risk allele for the PNPLA3 SNP rs738409 decreased significantly their body fat, DBP and insulin levels compared with those lacking a risk allele. MAST4HEALTH is an EU funded Marie Curie RISE program aiming at exploring the effect of Mastiha, a natural product of Greece which was shown to possess antioxidant/anti-inflammatory and lipid lowering properties. We designed a multicenter randomized double blind placebo controlled (parallel arm) clinical trial to test the effectiveness of Mastiha supplement for NAFLD/NASH treatment. MAST4HEALTH will explore gene-diet interactions, more specifically the potential personalized activity of the Mastiha, and correlate genetic and epigenetic markers with metabolomic and intestinal microbiota profiles pre- and post-intervention. The effectiveness of the proposed intervention will be evaluated via clinical and laboratory markers of the disease.

Xenohormetic nutrient signals that modulate aging and healthspan: a complex regulatory network for nutrigenomics research

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An extensive literature describes the positive impact of dietary phytochemicals on overall health and longevity. Although the exact mechanisms by which phytochemicals promote these effects remain to be elucidated, several reports have shown their ability to stimulate various mechanisms associated with aging process, including modulation of NAD+/sirtuin pathway and xenobiotic metabolising enzymes. Dietary phytochemicals include a large group of nonnutrients compounds from a wide range of plant-derived foods and chemical classes. Over the last decade, remarkable progress has been made to realize that chronic, low-grade inflammation and redox unbalance are critical aspects for the development of age-related diseases. Despite the translational gap between basic and clinical research, the current understanding of the molecular interactions between phytochemicals and oxy/inflammatory response could help in designing effective nutritional strategies to delay the onset of chronic diseases and improve healthy aging. Moreover, dietary phytochemicals have provided unique targets for underlying mechanisms of aging. Among these targets, SIRT1 has emerged as a good candidate to counteract oxidative stress and inflammation. Indeed, SIRT1 has several effects that may turn out to be a benefit given the multifactorial pathogenesis of aging and aging associated-disease. In this context, our and other laboratories, have highlighted the relevance of specific dietary phytochemicals to activate SIRT1 and also for the maintenance of the efficiency of the Nrf2/ARE pathway, a central mechanism for adaptive responses to oxidative stress and inflammation. This presentation will focus on the effects of some dietary phytochemicals on aging and longevity with particular focus on dietary patterns of long-lived populations but also on the importance of nutrient-sensing pathways that have a pivotal role in the regulation of oxi-inflammation and lifespan.
SELECTED ORAL PRESENTATION

Clinical Development of Tanibirumab and its next generation bispecific antibodies for cancer treatment

J.S. Yoo
PharmAbcine, Inc.

Vascular endothelial growth factor (VEGF) and its receptors are considered the primary cause of tumor-induced angiogenesis. Specifically, VEGFR-2/kinase insert domain receptor (KDR) is part of the major signaling pathway that plays a significant role in tumor angiogenesis, which is associated with the development of various types of tumor and metastasis. In particular, KDR is involved in tumor angiogenesis as well as cancer cell growth and survival. In this study, we evaluated the therapeutic potential of Tanibirumab, a fully human antibody against VEGFR-2/KDR. To assess the efficacy of the antibody and pharmacokinetic (PK) relationship in vivo, we tested the potency of TTAC-0001 in glioblastoma and colorectal cancer xenograft models. Antitumor activity of Tanibirumab in preclinical models correlated with tumor growth arrest, induction of tumor cell apoptosis, and inhibition of angiogenesis. We also evaluated the combination effect of Tanibirumab with a chemotherapeutic agent in xenograft models. We were able to determine the relationship between PK and the efficacy of Tanibirumab through in vivo single-dose PK study. I will discuss on our phase I and phase IIa data package of Tanibirumab.

Unbiased Mass-Spectrometry-Based Metabolomics Profiling in Over 1,000 Patients Reveals Specific Abnormalities in Biochemical Pathways in Patients with Atherosclerosis, as Defined by Cardiovascular CT-Based Precision Phenotyping in the Multi-Center GLOBAL Clinical Study

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Introduction: While the contribution of lipids and lipoproteins to atherosclerosis is well-described, little is known about specific derangements in biochemical metabolic pathways that exist in such patients.

Methods: We performed a nested case:control study in the broader GLOBAL multi-center clinical study (NCT01738828) in a total of 1,096 subjects (age 59.9 ± 0.55; 49% male) in three successive pre-defined cohorts. Since it is known that “time from last meal” affects metabolic profiles, we assessed differential metabolic profiles two different ways: 1. Fasting and non-fasting subjects combined and 2. Fasting subjects alone; fasting was defined as fasting for 8 hours or more. Validation was performed in a 3rd, independent set of fasting subjects. Non-targeted mass spectrometry (MS) analysis was performed by GC-MS and UPLC-MS/MS (Metabolon, Inc.; Raleigh, NC). Non-contrast and contrast cardiac CT was performed at each participating clinical site based on study protocol and all images were analyzed by independent core laboratory with consensus reads. “Case” was defined as ANY plaque on non-contrast/contrast CT; all others were controls. Univariate analysis with hierarchical clustering and multivariable analysis with gradient boosting was used for analysis; q-value of 0.05 or less (adjusted for false discovery rate) was significant.

Results: MS analysis quantified 1,088 analytes and of these, 83 and 34 were nominally associated with case:control status in the Discovery 1 and 2 Cohorts, respectively. Gradient boosting identified the 8 analytes that were strongest predictors, representing different biochemical pathways (Table).

Conclusions: Comprehensive metabolic profiling in the largest metabolomics study of atherosclerosis to date using cardiac CT as precision phenotyping tool identified simultaneous derangements in amino acid, pentose, nucleotide, dipeptide and anti-oxidant pathways; these provide mechanistic insights and provide potential diagnostic tools for human coronary atherosclerosis.

Single Nucleotide Polymorphisms in Dopaminergic pathways genes are associated with Bruxism

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Background: Bruxism (BRX) is a repetitive jaw-muscle activity characterized by clenching or grinding of the teeth and/or by bracing or thrusting of the mandible. BRX has two circadian manifestations, it can occur during sleep (SB) or during wakefulness (WB). However, it may be suffered together (W&SB). Mandibular movements observed in BRX have been compared to the movement patterns of Parkinson’s disease.
Theories that explain BRX etiology include alcohol consumption, smoking and alteration in neurotransmitters. Dopamine was proposed as a possible neurotransmitter associated with BRX. Genetic variants in Dopaminergic pathways (DP) are involved in Parkinson’s disease, alcohol consumption and nicotine dependence. Thus, there is a possible association of genetic variants in DP genes with BRX.

**Objective:** This investigation aimed to evaluate the frequency of genetic polymorphisms in DP genes (DRD1, DRD2, DRD3, DRD4, DRD5, MAOA and MAOB) in patients undergoing BRX treatment and a control group.

**Design:** Subjects submitted to BRX treatment were classified in WB (82 patients), SB (33 patients) and W&SB (63 patients). The control group included 135 patients. Polymorphisms of genes DRD1, DRD2, DRD3, DRD4, DRD5, MAOA and MAOB were evaluated using TaqMan® SNP genotyping assays (Applied Biosystem, USA).

**Results:** We found significant differences in genotypic frequencies for the following SNPs: rs10063995 of DRD1 gene (p < 0.0001), rs6280 of DRD3 gene (p = 0.036), rs6283 of DRD5 gene (p = 0.04), and rs1799836 of MAOB gene (p = 0.02). Moreover, we observed significant differences in the allelic frequencies of DRD1 rs10063995 SNP (p < 0.0001).

**Conclusions:** Our findings suggest a possible genetic contribution to the etiology of WB, SB and W&SB. Hence, it becomes necessary to continue research in this area to increase the current understanding of BRX physiopathology.

Friday 5 October, 2016

Session VII – PHARMACOGENOMICS AND PERSONALISED/STRATIFIED THERAPY

A novel predictive tool for 5-Fluorouracil toxicity and efficacy

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Background: 5-fluorouracil (5FU) is one of the most widely administered chemotherapeutic agents. A wide spectrum of toxicities can arise in treated patients, of which 10–30% experience severe to life-threatening adverse effects at standard doses (1). Factors affecting the extent of toxicity include sex, age, administration schedule, and activity level of enzymes involved in drug's action. DPYD gene polymorphisms, associated with low activity of the dihydropyrimidine dehydrogenase (DPD) enzyme, are accepted as a good predictive test to recognize patient at high risk of severe toxicity. Though, they can identify just a small percent of patients who will experience high-grade toxicity during 5FU treatment. Biochemical assay to evaluate the actual phenotype of 5FU metabolism can complement genotyping to increase specificity and sensibility of preemptive tests. We previously described an ex-vivo assay to measure individual 5FU degradation rate (5FUDR) in intact peripheral blood mononuclear cells (PBMC)

Objective: to evaluate the effectiveness of the 5FUDR assay, compared to DPYD genotyping, to predict efficacy and toxicity of 5FU treatments.

Design: 5FUDR and DPYD polymorphisms were analansyzed in a cohort of colorectal cancer patients and in a cohort of gastro-esophageal cancer patients and associated with outcome and toxicity of chemtherapy.

Results: low (< 5th centile) and high (> 95th centile) 5FUDR values were associated with severe 5FU toxicity in both the analyzed cohorts. The 5FUDR assay was able to identify an increased fraction of patients who developed severe toxicity compared to the DPYD genotyping.

Conclusions: 5-FUDR assay is a pre-treatment phenotypic test predictive of 5-FU severe toxicity and may be usefully implemented in clinical laboratory.

The use of Pharmacogenomic Methodologies in the Pharmacovigilance Evaluation of Medicinal Products

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Director Safety & Risk Management EAPA, Prahealthsciences

The presentation provides considerations on the influence of pharmacogenomics on Pharmacovigilance activities. This includes information on how to evaluate Pharmacovigilance related issues for medicinal products with pharmacogenomic associations and how to translate the results of these evaluations to appropriate treatment recommendations.

Due to gene-environmental interactions there exists large variability in responses to drug therapy, some of which are inherited or non-inherited characteristics of the genome. Such genomic variations have the potential to lead to subsets of patients with a different benefit/risk profile. It would therefore be important to consider genomic variations and explore the methods for collecting genomic data.

In the pre-marketing stage a medicinal product is exposed to a relatively small number of subjects due to the confines of the clinical trial. As such, rare and/or serious adverse drug reactions (ADRs) may only be identified later in the drug development process. Considerations should be made to identify sub-populations who may have increased or decreased sensitivity to medicinal products as a result of genomic factors. Doing so has the potential to greatly reduce the risk of side effects and significantly increase the therapeutic benefit to the subjects. During the process, and preparation, of risk management plans (RMP) it is essential to consider the potential risk of genomic variations and identify risk minimizations measures.

High Throughput ADME PGx Genotyping for Clinical Trial Stratification

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Pharmacogenomic (PGx) research on the absorption, distribution, metabolism, and excretion (ADME) properties of drugs has begun to have impact for both drug development and utilization. The current knowledge is advancing rapidly and genetic testing companies have to adjust to that. Currently there are many companies testing for Pharmacogenetics-related alleles but standardization is lacking. It is unclear what is being tested as well as how haplotyping is performed. This presentation will focus on the contribution that Agena Bioscience can provide through examples of participation in the Genetic Testing Reference Materials Coordination Program (GeT-RM) as well as through other comparison studies. It will also show Agena’s involvement in other pharmacogenetics studies and provide background on how the analysis platform works and can be used in a molecular diagnostic laboratory.
**GSTA1 genetic variants: a missing key factor in Busulfan first dose prediction models in conditioning before hematopoietic stem cell transplant in children**

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**Introduction:** Busulfan (Bu) is a key component of conditioning before hematopoietic stem cell transplant (HSCT) in children. Different predictive models have been used to predict Bu first dose and consequently drug exposure. The present study aims to evaluate the role of GSTA1 genotyping on performance of different models in predicting Bu first dose.

**Methods:** A hundred and twenty-nine patients who underwent an HSCT in our hospital between April 2002 and August 2015 after Busulfan-containing conditioning were included. Bu was given intravenously q6h (83) or q24h (46). Median of age was 5.5 (IQR 1.8 to 11.5) years, 48.1% were males and most patients had malignancies (67.2%). Most received BuCy (72.9%) as conditioning regimen. Administered first doses were calculated as previously published by Ansari et al. Pharmacokinetics (PK) parameters were assessed for all patients. Dose predictions were performed using 11 other published methods. Predicted Areas Under the Curve (AUC) were calculated assuming a linear relationship between predicted dose and predicted AUC. Target range for AUC was considered from 900μM.min to 1500μM.min. For clearance-prediction models, absolute prediction errors were calculated as follows: AE%=(CLpredicted-CLobserved)*100/CLobserved. Known polymorphisms of GSTA1 were analyzed at loci -1142, -631, -513, -69 respectively. Linear models were used to analyze GSTA1 genotyping as predictor of methods’ errors. Pearson’s correlation (r) between predicted and observed clearances was assessed.

**Results:** Mean administered dose was 0.85(0.10)mg/Kg for q6h doses and 3.51(0.56)mg/Kg for q24h doses, whereas overall predicted doses were 0.90(0.13)mg/Kg and 3.69(0.47)mg/Kg, respectively. A percentage of 29.1% of AUC’s were within the target for administered dose and 32.7% (range 7 to 53.5%) for predicted doses. Overall frequency of predicted AUC below, within and above the target were clearly impacted by GSTA1 haplotypes (64.3%, 24.1% and 8.6% in previously reported as good metabolizers, and 40.6%, 41.6% and 17.8% in poor metabolizers, respectively). At clearance prediction, models seemed to be similarly reliable (r from 0.83 to 0.9). Using McCune’s model, poor metabolizers had clearance well predicted, otherwise good metabolizers had prediction 28.4% lower than observed clearance (95%CI, 19.4-37.4%, p<0.001). In a multivariate model that included malignant vs non-malignant disease and gender, GSTA1 haplotype (p=0.004) and age (p=0.03) were significant factors to predict AE%.

**Conclusion:** GSTA1 haplotypes seem clearly to interfere in performance of dose prediction models in children. Adjustments of available models based on GSTA1 status or development of new population PK study including GSTA1 haplotypes are necessary to a more evenly distributed exposure among pediatric patients.


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**Session VIII – GENOMIC BIOMARKERS AND MANAGEMENT OF METABOLIC AND INFLAMMATORY DISEASES**

**Causality versus predictive ability in personalized medicine: C-Reactive Protein**

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Personalized medicine is a broad term, which narrows down practically to effective prediction, screening, diagnosis, and prognosis of complex outcomes in a by nature susceptible individual. Biomarkers, such as C-Reactive Protein (CRP), offer prevailing mandate to autograph the natural susceptibility of individuals and are used to classify individuals into homogenous groups for a disease/risk factors. So long in population based medicine, careful assessment of the validity of biomarkers has been essential in the era of disease prediction or the course of disease. However, this may not be sufficient in the era of personalized medicine, when critical decision beyond prediction for an individual or a patient, intervention and management will be made. Taking CRP as an example, this presentation puts forward the argument that in precision medicine, biomarkers with a causal association are perhaps more essential than those with only predictability.

**The gut microbiome and metabolic health**

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**Background:** In recent years, the increase in human microbiome research brought about by the rapidly evolving “omic” technologies has established that the balance among the microbial groups present in the human gut, and their multipronged interactions with the host, are crucial for health. The gut harbors the most dense and complex microbiota of the human body, which contributes importantly to several basic physiological functions, including nutrition, defense against pathogens and metabolic and immune homeostasis in the gut and beyond. Consequently, disturbances in the composition and function of the gut microbiota, i.e. dysbioses, can result in a broad variety of metabolic health problems.
**Objective:** Here, I review some of the complex relationships between gut microbiota alterations and metabolic health.

**Conclusions:** The gut microbiota is being established as an important factor in the regulation of host metabolism, mainly in relation to energy homeostasis and adiposity. The microbiota affects caloric intake, processing of polysaccharides, energy harvest, metabolic rate and fat synthesis and deposition. In particular, hepatic fat deposition may be stimulated by dysbioses through several mechanisms: regulation of gut permeability, modulation of bile acid and choline metabolism, and production of endogenous ethanol. In addition, the microbiota may influence numerous metabolic outcomes through inflammation-enhancing alterations. For instance, the microbiota has been shown to contribute to the chronic low-grade inflammation associated with an excess of adiposity that likely promotes the progression from obesity towards the metabolic syndrome. Similarly, influx into the portal vein of some bacterial components can promote the progression of fatty liver disease to steatohepatitis by enhancing hepatic expression of pro-inflammatory cytokines. In this respect, high fat diets induce an increase of bacteria containing lipopolysaccharides in the cell wall, resulting in higher serum levels of this pro-inflammatory molecule. A deficiency in the receptors that recognize specific microbe-associated molecular patterns, such as TLR5, also results in microbiota alterations that induce inflammation and metabolic syndrome conditions. Other main routes through which microbiota dysbioses will affect inflammation, as well as gut integrity, glucose homeostasis and fat deposition, are likely to involve the alteration of short-chain fatty acids production and of bile acids metabolism. For all these reasons, personalized medicine will need to take into account the composition and function of an individual’s gut microbiota in order to better prevent, diagnose and treat metabolic disorders.

### Interplay between epigenetic mechanisms in rheumatic disorders

S. Gay

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Our laboratory has been studying the epigenetic modifications over the past 15 years in inflammation in general and in particular in rheumatoid arthritis (RA) (1,2), progressive systemic sclerosis (scleroderma), pulmonary hypertension and ankylosing spondylitis. This work was supported by EC grants, including FP6 Autocure, FP7 Masterswitch, FP7 Osteoimmun, FP7 TEAM and the IMI 2 project BeTheCure (BTCure) The interplay of epigenetics is best illustrated by the involvement of multiple regulatory biological processes, such as acetylation, methylation, phosphorylation, sumoylation and non-coding RNAs, including microRNAs (miR), and long non-coding RNAs (lncRNA). Our center searches for all these epigenetic processes and how they interact (1).

Early studies have revealed that synovial fibroblasts (SF) in rheumatoid arthritis (RA) are hypomethylated and thereby activated to induce the MAPKinase pathway (2). Thereby, most recent studies on inhibiting specific enzymes in the polyamine pathway resulted in a novel therapeutic concept to silence the aggressive phenotype of RASF (3).

In related studies we could show that bromodomains 2, 3 and 4 are expressed in the RA synovium and can be targeted with selective BRD inhibitors to reduce the production of proinflammatory cytokines and matrix-metalloproteinases (MMPs) (4).

One of our greatest surprises in our current research has been the observation that SF differ in their phenotype depending from the localization in the body through the differential expression of miRs and lncRNAs (5). These findings are of fundamental importance for the homing of immune cells in health and disease.

**References**

### Contribution of an integrative multi-omic approach in the metabolic syndrome prediction: a nested case-control study

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**Background:** The rising worldwide prevalence of metabolic syndrome (MetS), a cluster of cardiometabolic risk factors of predictive of type 2 diabetes, relates largely to increasing obesity and sedentary but also to early metabolic life events [1].

**Objective:** The objective of the study was to identify predictive biomarkers of evolution toward MetS 8 years later, and to bring new knowledge about this pathological state using a multidisciplinary approach in an at-risk population (subjects with small birth weight).
Design: This case-control study (subjects free of MetS at baseline (n=92 born small vs n=76 born adequate for gestational age (SGA vs SGA)) was nested in the French community-based Haguenau cohort. The control group was randomly matched for age and sex. Serum signatures were determined and compared at baseline (20 years old) to determine predictive biomarkers using both untargeted mass spectrometry metabolomics and targeted proteomics using microarrays. Individual predictive models were first built using linear logistic regressions from the omics datasets. Metabolomic and proteomic data were finally integrated using random forest to determine whether multidimensional models improve prediction. Results: Univariate statistical analyses allowed identifying 93 discriminant metabolites and 47 proteins between cases and controls at baseline, with in both cases, specific gender differences. The resulting models based on either 4 metabolites or 4 proteins showed good performances: 22% misclassification on training set, 25% on validation set vs 11% misclassification on training set, 33% on validation set, respectively. Multi-omic data integration improved performance and robustness of the prediction (11% misclassification on training set, 8% on validation set). Correlation analyses with other data (anthropometric, biochemical) contributed to better understand the role of these biomarkers in the pathological processes, and therefore to evaluate their potential clinical value.

Conclusion: These results should provide new tools to better stratify at-risk populations, and additional knowledge on MetS development.

Acknowledgments: Project supported by the Fondation Francophone de Recherche sur le Diabète.

Reference

The association of ATP binding cassette gene B1 (ABCB1) C3435T polymorphism with apixaban peak concentration in patients with acute cardioembolic stroke and atrial fibrillation

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Background: Apixaban, an oral non-vitamin K anticoagulant, is a substrate for P-glycoprotein, which is encoded by ABCB1 gene. The single nucleotide polymorphism (SNP) (C3435T) in exon 26 of this gene correlates with the altered expression levels of P-glycoprotein, which may influence the pharmacokinetic parameters of apixaban. It was shown that plasma concentration monitoring could improve safety of non-vitamin K anticoagulants. Thus, data about genetic factors altering pharmacokinetics of apixaban would help to develop algorithms for anticoagulant therapy personalization.

Objective: The objective was to determine ABCB1 C3435T polymorphism association with apixaban peak concentration in patients with acute cardioembolic stroke and atrial fibrillation.

Design: 17 subjects with acute cardioembolic stroke and atrial fibrillation were enrolled. Apixaban administration was started on the 5th-12th day after stroke. Blood samples (6 ml) for pharmacokinetic and pharmacogenetic assessment were collected from an indwelling catheter or by direct venipuncture. Serial blood samples were collected prior to administration and 1, 2, 3, 4, 10 and 12 h after apixaban administration. High performance liquid chromatography mass spectrometry analysis was used to determine apixaban plasma concentration. Genotyping was performed by the CFX96 Touch Real Time System. The analysis was carried out by using the statistical software package IBM SPSS Statistics 20. Kruskal–Wallis and Mann–Whitney tests were used to compare the groups.

Results: Among 17 subjects 5 patients had CC genotype, 9 CT genotype, 3 TT genotype. Geometric mean Cmax of apixaban for CC genotype was 175,88 ng/ml, for CT genotype 97,01 ng/ml and for TT 148,21 ng/ml (р = 0,383). Geometric mean Cmax of apixaban for combined CC and CT genotype group was 119,98 ng/ml (р = 0,768).

Conclusions: We found no association between ABCB1 C3435T polymorphism and apixaban peak concentration in patients with acute cardioembolic stroke and atrial fibrillation.

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Session IX – NEW BIOMARKERS AND COMPANION DIAGNOSTICS

Cell-free DNA: The search of prognostic biomarkers in prostate cancer

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Unauthenticated
Background: Several studies have shown the potential role of cfDNA levels in the prognostic assessment of different solid malignancies. However, the quantification of pure cfDNA is a prerequisite for a reliable genotype analysis focused on the detection of cancer-specific DNA mutations signatures and/or epigenetic modifications. In this study, the quality and quantity of cfDNA were assessed by two different quantification procedures, furthermore cancer-specific DNA mutations as prognostic biomarkers in prostate cancer patients were tested.

Methods: A total of 25 prostate cancer patients and 30 aged matched healthy controls were enrolled into the study. Blood samples were collected at the diagnosis of prostate cancer, and at 6 and 12 months following the radical prostatectomy operation. cfDNA was extracted from plasma through Qiaagen kit and Promega automatic extractor. Qubit 2.0 was utilized for measurements of total amount cfDNA before qPCR quantification performed targeting of the single copy gene APP. Methylated GSTP1 and RASSF1A tumour specific cfDNA markers were determined.

Results: Preliminary data showed that patients with high cfDNA concentration at baseline had worse disease free time and overall survival.

Conclusion: The automated cfDNA extraction associated to the quantification by Qubit 2.0 seems to be the best approach to quantify the patient’s cancer-specific DNA mutations by qPCR assay. The combination of multiple mutational/methylation cancer biomarkers is suitable to determine the total amount of cfDNA in prostate cancer patients. cfDNA detection can be used as a prognostic and predictive tool for stratification, clinical management and follow-up of prostate cancer patients.

PCSK9 and Pharmacogenomics

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Abstract not available

Osteoporosis in the age of biotherapies with anti-sclerostin

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Osteoporosis is a worldwide public health concern. Osteoporosis has undergone extensive programs of fundamental and clinical research during the thirty past years. These programs have led to the creation of efficient pharmacological treatments of bone frailty.

Osteoporosis treatments may be divided in three groups:

- Bone metabolism repressors including vitamin D, estrogens and SERMs, bisphosphonates and, recently, RANK-RANK Ligand modulators (Denosumab)
- Bone metabolism activators including PTH 1-84 (Teriparatide) and sclerostin inhibitors. Anabolic steroids and fluorid salts are on back order in France.
- Bone metabolism uncoupling medications with strontium ranelate and cathepsin K inhibitors.

A huge amount of clinical data demonstrates the efficiency of osteoporosis treatments. The decrease of vertebral fracture risk reaches up to 50% for all treatments and up to 70% for parenteral treatments. Statistical analysis of data shows wide spread individual responses. A progressive bunch of evidence has demonstrated the need of applying modern criteria of predictive medicine to the treatment of osteoporosis.

Genome-wide association studies and whole-exome sequencing have identified genetic determinants of monogenic and complex conditions including osteoporosis and bone mass abnormalities. Overlap exists between the gene implicated in monogenic and complex forms of osteoporosis. Clinical discrepancies are largely explained by the clustering of gene encoding factors in signaling pathways crucial for osteoblastic and osteoclastic precursor cells differentiation.

Genetic and preclinical trials have focused on functional follow-up of many genes implicated in bone cell biology. The insight provided by genetic studies is serving the identification of biomarkers predictive of disease, redefining disease, response of treatment and discovery of new therapeutic targets for skeletal disorders.

The identification of genetic and molecular switches implicated in differentiation and maturation of osteoblastic and osteoclastic lineages has driven to the rise of innovative therapeutic tools. RANK Ligand modulators have reached clinical life. Anti-sclerostin and cathepsin K inhibitors reach the end of the clinical and regulatory pipe-line. Further developments in the field of bone biology and genetics might study canonic Wnt, DKK-1, Lrp-5 and later notch pathways. Interfering with all these genes must first take in account their tissue distribution. Gene modifiers may lack tissue specificity with major risk of adverse events.

Osteoporosis therapies fully belong to the new field of genomics and predictive medicine.
OneOmics in the cloud: integrating multi-omics data for quantitative system biology

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To build a model of a complex system, an understanding of how all the many different types of components of the system respond to a perturbation is needed. Powerful tools exist in each of the omics fields to quantitatively profile changes at the transcript level, the protein level, or the metabolite/lipid level. Often the expertise to perform these in-depth experiments lies in different labs or institutions, creating challenges for the integration of these separate disciplines.

There is a need to build tools and infrastructure that will allow integration of these projects and data streams. By bringing all this rich information together, we will have a truly systems approach to biological understanding that will drive translational medicine. The information gained by combination will be more powerful than that obtained from each omics technology in isolation.

To do this, we will need to be able to handle the combination of these massive datasets through more powerful informatics, more scalable computing power and enhanced usability so we can truly meet the needs of the biologist. This is all about OneOmics, a joint project from Sciex and Illumina.

Round Table – The future of personalised medicine, systems medicine and systems pharmacology – How to translate the big data for the clinicians and personalised therapy

Panel discussion
- Denis Horgan, EAPM, Brussels, Belgium
- Adrian Llerena, Badajoz, Spain
- Georges Dagher, Paris, France - Next generation Biobanking
- Magnus Ingelmann Sundberg, Stockholm, Sweden (Skype)
- Munir Pirmohamed, Liverpool, United Kingdom (Skype)
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A Phenome wide association study shows novel pleiotropic effects of the rs644234 on ABO gene

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Background: The Genome wide association studies (GWAS) have revolutionary improved discovery of novel genetic biomarkers determining genetic susceptibility to human diseases but the development of other methodological approaches is necessary in order to detect additional important information linked to the identified variants. Such an approach is the Phenome-wide association studies (PheWAS), which by analysing multiple phenotypes eventually linked to a single genetic variant lead to the identification of novel genotype-phenotype relationships and genetic pleiotropic discovery.

Objective: In this work we focused on the ABO gene, which produces the Histo-blood group ABO system transferase enzyme. This enzyme will modify the oligosaccharides of the glycoproteins, located in the cell surface, determining the type of blood group of one individual. Recent GWAS analyses have identified polymorphisms on this gene to be related with the thrombosis process, risk of suffering myocardial infarction and with multiple cardiovascular risk biomarkers. Especially the SNP rs644234, located in the intron 1 of the ABO gene, has been related with the levels of soluble E-selectin (sE-selectin).

Design: We performed a PheWAS analysis with the rs644234 and different inflammatory and lipid metabolism phenotypes available in the STANISLAS cohort, in order to investigate possible novel genotype-phenotype associations of the ABO polymorphism.

Results: The results showed pleiotropic effects of the rs644234. Indeed, we replicated previous known associations with sE-selectin (p-value: 6.6x10^{-10}), angiotensin I converting enzyme (ACE) (p-value: 0.008) and soluble intercellular adhesion molecule (sICAM) (p-value: 0.01). Moreover, new pleiotropic associations related with lipid phenotypes were found. The minor allele of rs644234 was associated with decreased ApoB (p-value: 0.019) and ApoE (p-value: 0.037) levels, and increased HDL levels (p-value: 0.012).

Conclusions: Previous GWAS studies have associated polymorphisms on ABO gene with the risk of suffering myocardial infarction, by increasing thrombotic risk as well as soluble adhesion molecules such as sE-selectin, ACE and sICAM. However, our results showed that the ABO gene has wider effects on lipid phenotypes, showing another pathway by which the ABO enzyme can impact on myocardial infarction. The pleiotropy showed for the ABO gene suggests that it has a complex role in the development of cardio-vascular diseases, and underline the importance of further studies in order to understand the exact mechanisms.

Using metabolomics to gain insight into major depressive disorder: a targeted approach on tryptophan pathway metabolites

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Background: Major Depressive Disorder (MDD) is a heterogeneous disorder with variable courses and responses to treatment, and for which the understanding of underlying biological mechanisms is still incomplete. Metabolomics could provide new insights on MDD mechanisms, as the metabolome reflects biochemical alterations in the pathophysiological state of biological systems. Tryptophan-derived metabolites raise particular interest, as an imbalance between neuroprotective and neurodegenerative metabolites in this pathway might be involved in major depression.

Objective: To investigate the modulation of tryptophan pathway metabolites in the plasma of MDD patients compared to healthy controls.

Design: We analyzed plasma samples from patients included in the METADAP study (a 6-month prospective, multicentric, real-world treatment study of 624 patients with a MDD diagnosis and a current major depressive episode) and from healthy volunteers (HV) who participated in the cross-sectional multicentric VARIETE study. A targeted UPLC-MS method was used to monitor 23 metabolites in the tryptophan pathway.

Results: Among the 14 metabolites that could be detected in the plasma samples, several were lower in MDD patients at inclusion (HDRS ≥ 18) compared to matched HVs, including serotonin, kynurenic acid, picolinic acid and indoxyl sulfate. Over METADAP study period (6 months of antidepressant treatment, during which a global decrease in the HDRS score was observed), plasma serotonin levels decreased, while the neuroprotective kynurenic and picolinic acids, as well as indoxyl sulfate, increased, approaching HV levels. Interestingly, the increase in kynurenic acid was more pronounced for the remitters than for the non-remitters.

Conclusions: these results support the hypothesis that an impairment in the neuroprotective components of the tryptophan pathway may be related to major depression.

Acknowledgements:
Effects of Mediterranean diet in obese patients with metabolic syndrome and depression

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Introduction: Central obesity is the source of inflammatory factors, causing endothelial dysfunction. In central nervous system, inflammatory factors mediate neurotransmitter metabolism promoting excitotoxicity, increasing oxidative stress, and major clinical symptom is depression. Mediterranean diet (MD) resulted as a good prevention method for depression.

Aim: To examine the relationship of depression with abdominal obesity and metabolic syndrome (MS) criteria, blood pressure, lipids, glycaemia and inflammation factors. Analyzing effects of MD on body weight, abdominal obesity and score points in Hamilton scale for depression.

Material and methods: Study included 36 adolescents and youth (16-30 years) and 22 adults over 30 years, overweight and obese patients with MS, diagnosed with depression using Hamilton scale. MS was diagnosed using ATP III classification criteria. The following parameters were observed: BMI, waist circumference (WC), blood pressure, lipids, CRP and basal glycaemia and insulin. Seven day scheduled MD was used by patients.

Results: Correlation is found between Hamilton scale score and body weight, BMI, WC (p<0.01) and CRP (p<0.05) in adolescents and youth. Implementation of MD in adolescents and youth resulted in reduction of body weight and WC (p<0.05), Hamilton scale score (p<0.001), insulinemia, HOMA-IR and CRP (p<0.5). Hamilton scale and Hamilton scale score correlated with insulin (p<0.05) and glycaemia (p<0.05), basal insulin correlated with CRP (p<0.05) in adults. After MD, values of Hamilton score, WC, insulin and CRP were lower (p<0.5) in adults.

Conclusions: Mediterranean diet resulted in reduced values of Hamilton scale score, waist circumference, insulinemia and CRP in patients with MS and depression. Positive correlation of Hamilton scale score with glycaemia and insulinemia suggest importance of insulin resistance and glucose regulation disorder on occurrence and stage of depression in obese patients with metabolic syndrome. CRP is useful marker of low grade inflammation in obese patients with MS and depression.

Keywords: metabolic syndrome, obesity, depression, Mediterranean diet, hyperinsulinism


The development of assays to evaluate Nrf2 regulatory mechanisms in cancer

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Background: Nrf2 is a cap’n’collar basic leucine zipper transcription factor that regulates the expression of antioxidant response element genes. These genes share a cis-acting enhancer where Nrf2 asserts its effect and regulates redox homeostasis, energy metabolism and DNA repair.

The activity of Nrf2 is maintained at a low expression levels under normal homeostatic conditions, but increases rapidly in response to redox, electrophile stressors and growth factors. Nrf2 is regulated independently by two ubiquitination pathways via interactions with either Keap1 or βTrCP. Interaction with the WD40 domain of the latter protein is mediated by DSGIS and DSAPGS motifs in Nrf2.

It has been widely proposed that Nrf2 plays a key disease-modifying role in conditions such as cancer, inflammatory conditions and neurodegenerative diseases. Therefore, there is interest in developing molecules that utilise Nrf2 as a therapeutic target.

Objective: The aim of this study was to identify mutations within the binding domain of βTrCP that can affect its interaction with Nrf2 and develop an in vitro assay to evaluate the interaction of βTrCP with small molecule and peptide ligands.

Design: Germline and somatic mutations were identified in the COSMIC, 1000 genome project, Ensembl, DisGeNET and MutDB databases. Fluorescence polarization and thermal shift assays were developed to rank ligands based on their IC50.

Results: 148 genetic variants were identified from the selected databases and scientific literature, of these 37% were synonymous, 60% missense and 3% stop-gained variants. Predictions of the consequences of amino acid substitution where performed using online tools. Molecular dynamics analysis was also performed to assess the impact of mutations on βTrCP-substrate interactions.

Conclusions: We have identified several mutations that may have a direct impact on the β-TrCP interaction with Nrf2 and therefore regulate its activity. The work has potential utility in understanding the role of germline and somatic mutations of βTrCP in various disease states and upon the feasibility of targeting βTrCP activity to modulate Nrf2 transcription in such conditions. Subsequently, by developing in vitro assays to study βTrCP interactions we can screen compound libraries that could enter pharmaceutical development.

Variant within the apolipoprotein B and its role in pseudo-FH development

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Background: Familial hypercholesterolemia (FH) is a serious disease, leading (if not treated) to premature myocardial infarction. FH is caused predominantly by mutations within the LDL receptor and APOB genes.

Objective: Recently, gene score from 12 SNPs within different genes have been defined, suggesting the existence of “pseudo-FH”, clinically indistinguishable from classical FH, but without one causal mutation. We have analyzed one from these SNPs in Czech patients with FH.

Design: APOB rs1367117 (G>A) variant was genotyped using TaqMan technology on an AB 7300 RT PCR cycler in 298 FH patients without the causal mutation and in 296 patients with the LDL receptor mutation.

Results: Frequencies of the individual genotypes significantly differ between two analyzed groups (P < 0.006). Minor A allele homozygotes occurs more often among the FH patients without the causal mutation than in patients with LDL receptor mutation (17.8% vs. 9.5%); with OR (95%CI) 2.10 (1.29 - 3.43; P < 0.003).

Conclusion: The results from our pilot study underline the importance of common APOB rs1367117 variant in the “pseudo-FH” development. Analysis of further SNPs is necessary to complete the real genetic causality of “FH” in patients without the detected mutation.

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The effect of acute Mastiha supplementation on the metabolites profile and oxidative stress in healthy humans

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Background: Mastiha is the natural resin harvested from the trunk of Mastiha tree (Pistacia lentiscus, var. Chia) growing exclusively in Chios Island, Greece. It consists mainly of terpenoids and polyphenols, with established antioxidant and anti-inflammatory properties (Paraschos et al., 2012), however the effect of its supplementation in a postprandial phase remains unexplored.

Objective: a) to assess the bioavailability of Mastiha’s phytochemicals in the plasma and urine of healthy subjects, b) to explore metabolites profile and metabolism mechanisms after Mastiha consumption using NMR and LC-HRMS based metabolomics c) to evaluate oxidative stress markers in a postprandial phase.

Design: Seventeen healthy non-obese and male volunteers, aged 20-40 years old, followed a low in phytochemicals diet for 5 days (e.g. no fruits, vegetables, legumes, coffee, cocoa, teas, wine). On the experiment day and after 12h fasting, volunteers consumed 10g of Mastiha powder dispersed in water. Blood samples were collected on time points 0h (before Mastiha ingestion) and 0.5h, 1h, 2h, 4h, 6h, 24h (post-ingestion). Urine samples were collected on time points 0h, 4h, 8h and 24h. Volunteers consumed nothing per os until time point 8h. Two different platforms, NMR & LC-HRMS were employed for the high throughput analysis of urine and plasma, respectively. Serum resistance to oxidation was measured with the CuSO4 technique. oxLDL and GSTp were measured in serum samples applying sandwich ELISA assays.

Results: Mastiha’s compounds were detected and identified in LC-HRMS profiles of plasma indicating that constituents such as moronic and isomasticadienonic acids are bioavailable. Also, multivariate spectroscopic data from NMR metabolomic profiles of urine samples were analyzed revealing certain clustering patterns of all the different time points. Moreover, several metabolites of triterpenic acids of mastiha were identified. Serum resistance to oxidation was measured as the difference (ΔT) of TLAG of each time point from TLAG 0h. Increase was statistically significant on time point 4h (402.3 ± 65.0sec) reached a peak on time point 6h (524.6 ± 62.9sec) and remained statistically significant until 24h post-ingestion (424.2 ± 68.0sec). oxLDL levels (expressed as % change from 0h), were reduced significantly from time point 1h until time point 8h post-ingestion. No significant changes in GSTp were detected.

Conclusions: It is evident that Mastiha exhibits metabolic alterations in healthy individuals and regulates markers of oxidative stress in the dynamic and unsteady postprandial phase. The present results provide new insights in understanding the metabolism pathways of Mastiha’s constituents.

Homocysteine- significant marker of metabolic syndrome and atherosclerosis risk- importance of si MS risk score

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**Background:** Obesity is followed by metabolic changes in form of insulin resistance (IR) and low grade inflammation. In patients with metabolic syndrome (MS) and clinically evident vascular complications homocysteine values are higher. Hyperhomocysteinemia correlates with hyperinsulinemia and IR, resulting in oxidative stress, which causes endothelial lesions and dysfunction, promoting hypertension and atherosclerosis.

**Objectives:** To examine homocysteine levels in patients with and without MS, and find correlation between factors of MS and homocysteine values.

**Methods:** The study included 76 obese individuals (age over 30, body mass index (BMI) >25 kg/m2) classified into two groups: I- with MS (35 patients); II- without MS (41 patients). OGTT was used to evaluate the extent of glucose regulation disorder. IDF classification was applied for diagnosing MS. Si MS risk score by Soldatovic I et al. was used. IR was determined by homeostatic model assessment (HOMA IR). Serum CRP was measured by immunometric assay. Microalbuminuria was determined immuno-nephelometrically. Homocistin was determined by immunoassay FPIA-Abbott.

**Results:** Patients with MS had increased WC: (I-110.6±15.4, II±15.4 cm), BMI: (I-35.3±6.6, II-30.4±7.4 kg/m2), blood pressure (I-136.4±13.9 /90.5±9.5, II-118.4±12.7/78.1±9.7 mmHg), glycaemia (I-5.4±1.6, II-4.8±0.8 mmol/l), HOMA IR (I-8.8±9.4, II-5.2±3.8 mmol/l/μU/ml), triglycerides (I.217±0.95, II-1.45±0.7mmol/l), CRP (I-0±0.0, II- 3.7±3.8mg/l), microalbuminuria (I-87±81, II-56.4±56.9mg/24h), homocysteine (I-13.0±3.2, II-11.8±3.7 (μmol/l) and decreased HDL (I.0±0.2, II-1.35±0.35mmol/l). Statistical significance between groups was found for WC, BMI, systolic and diastolic pressure, triglycerides, HDL-cholesterol (p=0.01) and CRP, Apo B, HOMA IR (p=0.05). Correlations: Homocysteine with systolic and diastolic pressure, Apo B and hyperlipoproteinemia (p=0.05). Si MS risk score with homocysteine (p=0.01), r=0.263.

**Conclusion:** Abdominal obesity, hypertension, hypertriglyceridemia, inflammation factors, IR, homocysteine and microalbuminuria as markers of endothelial dysfunction were increased in patients with MS. Correlation of homocysteine values with si MS risk score indicates that it is significant co-founding factor of MS. Correlation of homocysteine with hypertension and hyperlipoproteinemia indicates importance of homocysteine as significant marker for atherosclerosis.


Risk factors for NODAT under immunosuppressants in renal transplanted children

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**Background:** New Onset Diabetes After Transplantation (NODAT) is a major complication of immunosuppressive therapy and a risk factor for transplant rejection. Its incidence ranges from 4 to 25% in adults. In 2013, L. Elens and coworkers reported the association of two genetic variants POR*28 and eA42 with the occurrence of NODAT in renal transplanted children treated with tacrolimus, MMF and corticosteroids.

**Objective:** Our objective was to assess the impact of these two genetics variants along with additional risk factors (age, sex, HLA mismatches…) on the occurrence of NODAT among renal transplanted children treated with tacrolimus, MMF, and corticoids.

**Design:** Two hundred thirty eight renal transplanted children were identified between 1990 and 2013 in Robert Debré hospital. After DNA extraction, genotypes were determined using allelic discrimination technique TaqMan. The statistical analysis have been performed using SPSS v15.

**Results:** One hundred and nine renal transplanted children were treated with tacrolimus and were included in the study (63 boys/46 girls). The mean age at time of transplantation was 10.8 years (± 0.5) and mean duration of follow-up was 4 years (± 0.3). A significant association of NODAT was evidenced with POR*28 (p=0.010), number of HLA mismatches (p=0.004) and donor’s CMV serology (p=0.026) but not with PPARα (p=0.434).

**Conclusions:** This pilot study validated the association between the SNP POR*28 and the occurrence of NODAT in renal transplanted children treated with tacrolimus. Early identification of patients carrying POR*28 genetic variant might allow to prevent the risk of NODAT and improve patient’s care.

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Impression cytology of the conjunctival epithelial cells in patients with Graves orbitopathy

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Background: Graves’ orbitopathy (GO) is rare in pediatric patients, however is the most common extrathyroid manifestation of Graves’ disease (GD), being present in 30-67% of patients. GO is an autoimmune inflammatory disorder involving orbital connective and fatty tissues as well as the extraocular muscles. In children, GO is less common and less severe than in adults. The most common symptoms are upper eyelid retraction, conjunctival injection, and proptosis and periorbital edema. Severe complications include dysthyroid optic neuropathy, corneal ulceration and eyeball subluxation.

Objective: The aim of this study was evaluating goblet cell population and conjunctival epithelial morphology in patients with GO.

Design: Twenty GO patients (9 males and 11 females, age range = 7 - 23 years) and twenty controls (10 males and 10 females, age range = 8 - 22 years) underwent conjunctival impression cytology. Conjunctival epithelial cells were analyzed microscopically, with regard to their shape, size, the nucleus-to-cytoplasm ratio and nuclear chromatin condensation using an Olympus Bx50 light microscope.

Results: Impression cytology showed conjunctival squamous metaplasia and goblet cells loss in patients with GO.

Conclusions: Reduced goblet cells numbers and squamous metaplasia may be indicative of a higher degree of epithelial damage of conjunctival epithelial cells in GO patients, and the presence of lymphocytes and neutrophiles is a strong sign for an inflammatory background of this disease. In view of the simple, noninvasive nature of impression cytology, this technique may prove to be an important tool for the diagnosis and monitoring of dry eye changes in GO patients.

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Increased Sleep Bruxism Risk is Associated with the HTR2A rs2770304 SNP

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Background: Bruxism (BRX) is a condition of interest for researchers and clinicians in both dental and medical areas. BRX has two circadian manifestations, it can occur during sleep (sleep bruxism; SB) or during wakefulness (awake bruxism; WB). However, it can be suffered together as well (SB&WB). Researchers suggest that central nervous system neurotransmitters and its related genes could be involved in the genesis of BRX. Serotonin is responsible for the circadian rhythm, maintaining arousal, regulating the stress response, muscle tone and breathing. Thus, serotonin could be associated with the pathogenesis of BRX.

Objective: We aimed to evaluate the frequency of genetic polymorphisms within HTR1A (rs6295), HTR2A (rs1923884, rs4941573, rs6313, rs2770304), HTR2C (rs17260565) and MAOA (rs12843268) genes in patients undergoing Bruxism treatment.

Design: Were included 179 patients submitted to BRX treatment, who were classified according to their diagnosis in SB (33 patients), WB (82 patients) and SB&WB (63 patients). The control group included 135 patients. Genomic DNA was extracted from blood leukocytes using the method described by Salazar et al. (2001). Genotypes of HTR1A, HTR2A, HTR2C and MAOA were evaluated using TaqMan® SNP genotyping assay (Applied Biosystem, USA), in a StepOne™ Real-Time PCR system (Applied Biosystem, USA).

Results: We found differences in allelic frequencies for the HTR2A rs2770304 SNP. The C allele was associated with increased SB risk (odds ratio = 1.96, 95% confidence interval: 1.34 – 3.39, p=0.02, Fisher’s exact test).

Conclusions: Our results indicate that the HTR2A rs2770304 SNP is involved in SB. Therefore; it becomes necessary to continue exploring the genetic basis of circadian manifestations of BRX to increase the current understanding of BRX physiopathology.

Global DNA methylation is associated with circadian manifestations of Bruxism

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Background: Bruxism (BRX) is defined as a repetitive jaw-muscle activity characterized by clenching or grinding of the teeth and/or by bracing or thrusting of the mandible, having two main circadian manifestations; it can occur during sleep (SB) or during wakefulness (WB). However, it may be suffered together (W&SB). Relatives of people suffering BRX are also affected by this parafunction, therefore, possible genetic and/or epigenetic factors may be involved. BRX etiology includes alcohol consumption, smoking and neurotransmitters alterations. In addition, DNA methylation is a physiological epigenetic modification, occurring primarily by the addition of a methyl group to a CpG dinucleotide in a DNA sequence that regulates gene transcription. Altered global DNA methylation content is a feature of several diseases, and aberrant methylation is considered a mechanism by which environmental risk factors (e.g., tobacco, alcohol, and diet) may influence disease risk. Thus, circadian manifestations of BRX could be associated to aberrant DNA methylation.

Objective: We aimed to quantify global DNA methylation in patients under BRX treatment and controls.

Design: Subjects undergoing BRX treatment were classified in WB (42 patients), SB (32 patients) and W&SB (42 patients). The control group included 42 healthy patients. To quantify global DNA methylation, we used the ELISA kit MethylFlash Methylated DNA Quantification Kit (Colorimetric assay, Epigenetic Group Inc., NY, USA).

Results: We found significant differences in the content of methylated DNA in all circadian manifestations of BRX compared with the control group (SB 0.95 ± 2.02 ng/μL; WB 0.87 ± 2.1 ng/μL; W&SB 0.17 ± 0.25 ng/μL; Control 1.69 ±1.6 ng/μL; Kuskal-Wallis test [p = 0.0001] followed by Dunn’s test [p <0.05]).

Conclusions: DNA in BRX patients was hypomethylated compared with the control group. Our results suggest that DNA methylation might be a novel etiologic factor involved in BRX etiology. Further research must be performed exploring the role of epigenetics modifications in circadian manifestations of BRX.

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CLOCK GENES AND SLEEP DISTURBANCES IN AUTISM SPECTRUM DISORDER

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Introduction: Subjects with Autism Spectrum Disorder (ASD) have the circadian cycle affected. Sleep-related problems (SRP) affect up to the 84%. The molecular mechanisms of the circadian cycle involve clock genes and melatonin route genes. SRP encompass a wide range of conditions that affect to sleep parameters and have been categorized in the International Classification of Sleep Disorders.

Aim: Evaluate the relationship between SNPs in ASMT, Perl, Npas 2, and MTNR1A genes and SRP tracked with actigraphy.

Methods: A case control study was performed in subjects with ASD (n=25, 80% 7 years, mean age of 32±2 years) matched with controls (n=26, 62% 9 years). Sleep parameters (Sleep Onset Latency (SOL), Sleep Efficiency (SE) and Total Time Sleep (TTS)) were measured by actigraphy and sleep diaries during a week. DNA was extracted from peripheral blood and genotyping of the ASMT (rs444690 and rs5989681), Perl (rs6416892 and rs885747), Npas2 (rs1811399), MTNR1A (rs28383652) genes was performed by Multiplex PCR.

Results: Significant differences (p< 0.05) were found in sleep parameters from ASD compared to controls (SOL 67±14 vs 17±2 minutes; TTS 465±100 vs 404±58 minutes and SE 79% vs 93%). Recessive genetic model from rs5989681 and overdominant genetic model from rs6416892 and rs885747 show a relation between genotype and SOL (p = 0.04; p = 0.02 and p = 0.02).

Conclusions: Actigraphy is an objective and useful tool to analyze SRP in ASD population. Apparently, there is a relation between SOL Perl and ASMT, but further genetic analysis is required.
Multi-omics, multi-tissue association study identifies novel epigenetic candidate loci and genomic structural variants implicated in dilated cardiomyopathy and heart failure

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Background: Dilated Cardiomyopathies (DCM) can be caused by single nucleotide variations (SNV) in one of more than 40 different genes. Besides SNVs and small coding insertions and deletions, it is postulated that epigenetic modification as well as larger structural variants (SV) could be involved in the pathogenesis of DCM.

Objective: The objective was to identify novel susceptibility regions both on the structural genomic and epigenetic level that are linked to myocardial dysfunction and heart failure as well as potential biomarker candidates.

Design: To elucidate the contribution of structural variants to DCM, we performed whole-genome sequencing in a cohort of n=50 consecutive patients with primary DCM. Additionally, we performed mRNA transcriptome sequencing and DNA methylation profiling using the Illumina 450k chip of the same myocardial specimens of the left ventricle and from whole blood in each patient and control (n=31).

Results: Using a stringent multi-omics, multi-tissue approach and by validation in independent samples, a set of high-confidence candidate loci that are epigenetically regulated in DCM could be identified. Besides known disease-causing point mutations in 12 patients, we also detected 3,898 genomic structural variations. Additionally, we could show the functional effect of SVs on important cardiac genes and novel DCM candidates.

Conclusions: The present study provides to our knowledge the most comprehensive mapping of DNA methylation in human heart tissue and identifies novel loci associated with heart failure and DCM using a comprehensive approach covering genetic variation, DNA methylation and whole-transcriptome analyses. Additionally, we demonstrate the effect of SVs on cardiac gene transcription and its association with human Dilated Cardiomyopathy. It will be interesting to dissect large-scale cohorts to define exact frequencies of SVs that we could link to human Dilated Cardiomyopathy for the first time as well as to validate the identified epigenetic biomarker candidates.


Metabolomics as a new key player in systems biology for better understanding nutrition-health relationship

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Background: Human body responses to nutrition and chronic metabolic disorders represent complex features that require integrated systems biology approach to better understand their relationship. Metabolomics, as a powerful phenotyping tool offers now the possibility of characterizing global alterations associated with nutritional exposure and disease conditions, and of identifying early and/or predictive biomarkers of disease development [1].

Objective: The objective of this work was to perform a proof of concept study of the use of untargeted metabolomics as part of an integrated strategy for identifying predictive biomarkers of the evolution toward Metabolic Syndrome (MetS), and bring new knowledge about this pathological state.

Design: A nested case-control approach was used within the French GAZEL cohort (n=20,000): at risk male subjects (n=112, 54–64 years old) with high body mass index (BMI, 25 ≤ BMI < 30), free of MetS at baseline, were selected. Cases who developed MetS at the follow-up (5 years later) were compared with Controls (matched for BMI and age) for several parameters (clinical, biochemical parameters, and food habits). Baseline serum samples were analyzed using mass spectrometry-based untargeted metabolomics. A full bioinformatics workflow was optimized to process the data. In particular, data mining methods were used to select the best candidate for prediction. Metabolomic data were then integrated with the different parameters from the database to determine whether multidimensional models improve prediction and impact subject stratification.

Results: Metabolomic data allowed improving prediction capacity compared to the use of clinical data only. The multidimensional model integrating metabolomics with anthropometric, biochemical and nutritional data showed the best prediction performances. The analysis of the misclassified subjects revealed sub-phenotypes and generated hypotheses about the disease associated feature space, that need further characterization in order to propose adequate patient sub-stratification and prevention.

Conclusion: Metabolomics has the potential to become a key player in systems diagnosis strategies. Integration of metabolic profiles into multidimensional models can be an essential tool for a patient-centered personalized prevention.

Role of *Enterococcus faecalis* in endodontic treatment prognosis of devitalized teeth

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**Background:** *Enterococcus faecalis* (EF) has been associated with endodontic treatment failure (ETF) in devitalized teeth. Antimicrobial resistance is a worldwide issue. Has been reported that EF would be resistant to medications used into root canals (RC) during endodontic treatments. Thus, EF has the ability to remain in quiescent state into RC. Researchers suggest that these characteristics are due to the expression of certain genes and the operation of a proton bomb. The ability of EF to survive in unfavorable environments and in presence of intracanal medications, support its role in ETF. Thereby, is important to know the prevalence of EF in teeth that needs an endodontic treatment.

**Objective:** To determine the presence of EF in devitalized teeth with endodontic treatment indication.

**Design:** Were obtained 85 samples of RC of devitalized teeth from dental teaching clinics of Universidad Mayor, Temuco, Chile and from a private dental clinic in Temuco, Chile. The samples were classified according to diagnosis in dental pulp necrosis (DPN), periapical granuloma (PG), acute periapical abscess (APA), chronic periapical abscess (CPA) and endodontic failure. Bacterial culture was performed using Enterococccol agar medium and Potassium Tellurite medium.

**Results:** The pulpal diagnosis of teeth treated in University were different compared with treatment performed in the private clinic (p=0.01, chi-square test), DPN and PG were more frequent in the University (p=0.007, Fisher’s exact test) and CPA was more common in the private clinic (p=0.003, Fisher’s exact test). The highest frequency of EF was obtained in PG, and the lowest in APA (p=0.035, chi-square test).

**Conclusions:** EF is associated to PG diagnosis. An effective elimination of EF is necessary prior to RC filling, to reduce the risk of ETF. Further researches must be performed to develop more effective intra-canal medications. Besides, is mandatory to determine the mechanisms involved in resistance of EF to medications used in endodontics.

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Different distribution of dopamine receptor D2 (DRD2) gene polymorphisms between smokers and drug dependent subjects among Malay male population in Kelantan, Malaysia

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**Background:** Drug addiction and cigarette smoking remain worldwide problems, and their negative impacts on society are increasing. A common pathway for the reinforcing properties of most addictive drugs is the mesolimbic brain dopamine system. The system has also been implicated in the reinforcement of the effects of nicotine in smoking. The systems also have been shown to be involved in the reward pathway of drugs of abuse. One of the genes, dopamine receptor D2 (DRD2) gene has been reported to be one of the plausible candidate genes for nicotine dependence as well as genes involved in the rewarding effects of drugs of abuse.

**Objective:** The objective of the present study was to compare the allelic frequencies and genotypes of DRD2 gene polymorphisms between smokers and drug dependent subjects among Malay male population in Kelantan, Malaysia.

**Design:** 130 Malay male smokers and 100 Malay male drug dependent subjects were recruited. All subjects were given informed consent form to participate in the study. The study was approved by Ethics Committee of School of Medical Sciences, Universiti Sains Malaysia. DNA was extracted from leucocytes and the allele was determined by a polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). The PCR product was digested with restriction enzymes *TaqI*. The statistical analyses was performed using the SPSS package version 20.0.

**Results:** Interesting difference in gene distribution between the two groups was discovered. 49% of the smokers were found to have homozygous DRD2 A1/A1 genotype while 55% of drug dependent subjects were found to have heterozygous DRD2 A1/A2 genotype. There existed significant differences both in the frequencies of alleles and genotypes between subjects.

**Conclusions:** To conclude, it appears that there was a difference in gene distribution of dopamine receptor D2 (DRD2) between smokers and drug dependent subjects among Malay male population in Kelantan, Malaysia

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**Reference:** 1. Missale C, Nash RS, Robinson SW, Mohamed J, Marc GC. Dopamine receptors: from structure to function. Physiol Rev 1998; 78: 189-225
MiRNAs as potential salivary biomarkers in periodontitis

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Background: MiRNAs are small noncoding-RNAs that regulate gene expression. Their expression is frequently altered in disease, translating into distinct miRNA expression profiles. MiRNAs are present in extracellular fluids such as saliva, where they also correlate with various physiopathological conditions. Specific salivary miRNA signatures could thus be defined and used as risk, diagnostic, prognostic or treatment response biomarkers.

Objective: Through systematic literature review and a computational approach, we aimed to define a comprehensive set of miRNAs with potential use as salivary biomarkers for periodontitis. Design: PUBMED and Web-of-Science databases were searched using the expression (MicroRNA* OR mirna* OR miR) AND (Periodont* OR Gingiv* AND Inflam*). Additional articles were selected from the reference lists. The resulting 19 studies were searched for miRNAs with differential expression (p<0.05 on qRT-PCR) in periodontitis patients or cell cultures (upon bacterial stimulation). Presence in saliva was checked from an external database. MiRWalk was used to identify periodontitis-associated validated targets and other miRNAs targeting periodontitis-associated genes.

Results: 42 miRNAs were differentially expressed, 36 of which had validated periodontitis-associated targets. All but one were previously detected in saliva. hsa-miR-146a-5p was the most promising candidate: it fulfils all three criteria and is differentially expressed in all types of studies, with agreeing replication. Consistent evidence also exists for hsa-miR-146b-5p and hsa-miR-223-3p. 406 other miRNAs are present in saliva and target other periodontitis-associated genes: 37 of these have 4 or more validated periodontitis-associated targets.

Conclusions: We provide a comprehensive set of miRNAs that 1) are differentially expressed either in periodontitis or in cell cultures upon bacterial stimulation, 2) are present in saliva and 3) target periodontitis-associated genes. Validation of these miRNAs as salivary biomarkers for periodontitis will require further studies to confirm differential disease-associated salivary expression and to assess the corresponding sensitivity and specificity in clinical context. Translation of salivary biomarkers to clinical practise will allow the development of fast, noninvasive and inexpensive omics-based screening tests for early diagnosis and personalised periodontal management.


Identification of miRNAs with potential utility as salivary biomarkers for type II diabetes

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Background: MiRNAs are small noncoding RNAs that mediate post-transcriptional gene expression regulation. They play a central role in diverse biological processes and may be present in extracellular fluids such as saliva. Since the spectrum of salivary miRNAs correlates with physiopathological state, well-defined panels of salivary miRNAs could be used as risk, diagnostic, prognostic or treatment response biomarkers. Salivary miRNAs are therefore attractive for clinical application.

Objective: Through systematic literature review and a computational approach, we aimed to define a set of miRNAs to be evaluated as potential biomarkers for type II diabetes mellitus (T2DM).

Design: PUBMED and Web-of-Science databases were searched using a combination of the MeSH terms “Diabetes Mellitus, Type2” and its synonyms AND MicroRNAs OR Insulin resistance OR Hyperinsulinenia OR Glucose intolerance OR Hyperglycaemia. 776 articles were retrieved, 31 of which complied with predefined inclusion/exclusion criteria. MiRNAs differentially expressed between T2DM patients and normal glucose tolerance (NGT) controls (p<0.05 on qRT-PCR validation) were identified, excluding conflicting results. An external database was used to check for miRNAs presence in saliva and miRWalk was used to identify T2DM-associated validated targets of such miRNAs. MiRWalk was also used to identify other salivary miRNAs targeting T2DM-associated genes. Results: 49 miRNAs were differentially expressed in T2DM. Among these, according to miRWalk, 19 have a T2DM-relevant disease ontology annotation, regulating 11 different genes. All but one of these miRNAs were previously detected in saliva, making them suitable as salivary biomarkers. 162 other salivary miRNAs also target T2DM-associated genes: 21 of these have 2 or more validated T2DM-associated targets.

Conclusions: We provide a comprehensive set of miRNAs that are differentially expressed in T2DM patients, target T2DM-associated genes and were already detected in saliva. Validation of these miRNAs as salivary biomarkers for T2DM will require further studies to confirm differential salivary expression in T2DM and to assess sensitivity and specificity in clinical context. Translation of saliva-based biomarker assessment to clinical practise will allow the development of fast, noninvasive and inexpensive omics-based screening tests for early diagnosis and person-alised T2DM management.

The Study of Protective Effects of Grape Seed Extract Against Damage of the Organism Caused by Heavy Metals in *Rattus Norvegicus*

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**Background:** *Vitis vinifera* seeds contains very high content of antioxidants [1]. They have a higher antioxidant potential than common kind of fruits and vegetables. Cadmium (Cd) is one of the most prevalent toxic metal.

**Objective:** The study explored the protective effects of grape seed extract (GSE) against Cd toxicity in the organism of *Rattus norvegicus*.

**Design:** Male adult *Wistar* rats (n=16) were divided into four equal groups. The first group was control, the second was treated with GSE (0.5 g of lyophilized powder/rat/per day), third was treated with CdCl₂, (10 mg CdCl₂/rat/per day), and fourth CdCl₂ (10 mg CdCl₂/rat/per day) plus GSE (0.5 g of lyophilized powder/rat/per day). Content of Total Bilirubin, Liver Enzymes, Antioxidant enzymes and Albumin were increased in the Cd groups. The results showed that Cd could increase oxidative stress. Among the most significant indicator appears ALT, AST, GPx and SOD where group with CdCl₂ had more than 3 times higher content in blood. GSE had a protective effect itself, results indicate decreased levels of antioxidant enzymes in the blood of *Rattus*. Add Cd together with GSE caused, that GPx and SOD was only 50% higher than control group.

**Conclusions:** This study concluded that GSE has beneficial protective effects against damage of the organism caused by heavy metals in *Rattus norvegicus*.

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A multimarker approach for the prediction of 30-day major adverse cardiac events in patients with ST-segment elevation myocardial infarction: cost-effectiveness analysis

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**Background:** Despite advances in treatment, acute coronary syndromes, which consist mainly of ST-segment elevation myocardial infarction (STEMI) and unstable angina/non-STEMI, present an enormous medical, social, and economic burden worldwide. Primary percutaneous coronary intervention (pPCI) is a therapy of choice for the management of patients with acute STEMI. Despite the improvement in morbidity and mortality in these patients, groups at high risk of complications and adverse clinical events remain. Identification of patients at risk for major adverse cardiovascular events (MACE) might help selecting candidates for aggressive treatment which may improve outcome or early discharge after pPCI. With expansion of the number and types of circulating biomarkers available, the opportunity to improve risk stratification continues to grow.

**Objective:** To explore discriminative abilities of several biomarkers of inflammation and hemodynamic stress as predictors for MACE in patients with STEMI treated by pPCI. Also, to assess their cost-effectiveness compared with the RISK-PCI score for the prediction of MACE during a 30-day follow-up after pPCI.

**Design:** Using a decision model, the costs, accuracy, and cost-effectiveness of each model was evaluated. The RISK-PCI score was used as the baseline model. Other models were formed with the consecutive addition of selected markers: myeloperoxidase (MPO), high sensitive C-reactive protein, interleukin-6, adiponectin, oxidized LDL, B-type natriuretic peptide (BNP), N-terminal-proBNP to the baseline model. A best-case model was formed from a combination of biomarkers to yield the best patient stratification algorithm. All models were assessed by their predictive probabilities using receiver operating characteristic curves. One hundred fifty STEMI patients treated by pPCI was included in the study. Composite 30-day MACE was defined as cardiac death, non-fatal reinfection, and target vessel revascularization. The analysis was performed from a third-party payer perspective.

**Results:** Only two strategies had outstanding discriminative abilities: the best-case model (RISK-PCI score+BNP+MPO) and RISK-PCI score plus BNP with area under the curve (AUC) values of 0.869 and 0.851, respectively. The cost-effectiveness ratio varied between 5199 € per AUC for the baseline model to RSD 9011€ per AUC for RISK-PCI score+NT-proBNP. After elimination of dominant strategies, the incremental cost-effectiveness ratio (ICER) for the remaining three strategies (baseline, RISK-PCI score plus BNP, and the best-case model) were calculated. For the RISK-PCI score plus BNP, the ICER (compared with the baseline model) was 18106 € per additional accuracy calculated for 100 analyses. The ICER for the best-case model (compared with the baseline model) was 84961€ per additional accuracy calculated for 100 analyses. Strategy involving hemodynamic stress biomarker BNP was more cost-effective than strategies involving inflammatory markers. Sensitivity analysis indicated that results were robust.
Conclusion: The obtained results support the feasibility of a multimarker approach for MACE prediction in STEMI patients treated by pPCI. The introduction of BNP in the clinical laboratory would be convenient and cost-effective.


The VEGF Consortium
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Background: The Vascular Endothelial Growth Factor (VEGF) is a pivotal mediator of angiogenesis, known to impact both on physiological processes and on the development of complex chronic diseases. The Vascular Endothelial Growth Factor European Genomic Federation - VEGF Consortium (http://www.vegfconsortium.org aims to develop a transnational collaborative network dedicated to large integrative and multidisciplinary genomic studies on VEGF in order to generate actionable knowledge for medical practice of chronic diseases.

Objectives: To identify VEGF ‘-omics’ profiling in health and disease to be used for patient stratification.

Design: Fourteen partners from 8 countries are involved in the consortium, representing ten internationally recognized cohorts totaling >15000 individuals. These partners are among the worldwide leaders in the fields of genomics, transcriptomics, epigenomics, “-omics” methodologies and analyses, chronic diseases prediction and prevention, personalised medicine, personalised therapy, pharmacogenomics, clinical implementation, and communication.

Results: Through two Genome-Wide Association Studies, we have identified 10 genetic variants, collectively explaining up to 50% of VEGF phenotypic variation1, 2. We have also demonstrated associations between these polymorphisms and cardiovascular (CVD) risk factors such as blood circulating lipid levels, as well as epistatic (gene×gene) interactions. Eleven working groups have been formed.

Conclusions: The VEGF consortium brings together a large number of high-level researchers and academics, along with their expertise and resources. It is expected to provide robust results on the role of VEGF in chronic diseases prediction, prevention and personalized therapy. It is an original concept open to further collaborations in order to advance research on this challenging field.

References:
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SERT, DAT1 and COMT Polymorphisms in Psychiatric Patients

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Background:
Serotonin transporter is connected to serotonergic neurotransmitter pathway. Best studied is the promoter region polymorphism, 5-HTTLPR, a repetitive element of varying length (short or S allele, or long or L allele) in the promoter of SLC6A4. S allele was shown to be associated with a variety of emotional disorders and psychopathological traits such as depression, suicidality, bipolar affective disorder and some others, but the findings were inconsistent.

On the other side, dopaminergic pathway indicators connected with psychiatric disorders include Dopamine Transporter (DAT) and Cat-echol-O-methyl Transferase (COMT). DAT transports dopamine into presynaptic neurons, while COMT is involved into dopamine degradation. There are two most investigated polymorphisms which were reported to affect dopaminergic pathway and psychiatric disorders: DAT1 VNTR (rs28363170) and COMT Val158Met (rs4680). Research of DAT1 VNTR showed relationship of 9 or 10 repeats allele with higher gene expression levels that leads to higher DAT protein availability. COMT Val158Met polymorphism showed to be related to higher levels of dopamine (Met allele).

Objective: As research results of genetic polymorphisms for all three genes were found to be inconsistent across literature, when trying to relate them to psychiatric disorders, we tried to investigate these relationships in context of our healthy subjects and patient’s data diagnosed with depression and schizophrenia.

Design: We compared genetic polymorphism distribution in healthy subjects vs patients diagnosed with depression and schizophrenia for three genes: 5-HTTLPR, S/L polymorphism (Healthy subjects=303, Depression=298), DAT1 VNTR, alleles 6, 9, 10, 11 (Healthy subjects=295, Schizophrenia=303) and COMT, Val158Met (Healthy subjects=306, Schizophrenia=299)

Results: For 5-HTTLPR, S/L polymorphism when compared depression vs schizophrenia vs control group we found 60(38%) vs 118(40%) vs 105(35%) LL genotypes; 81(42%) vs 120(40%) vs 145(48%) LS genotypes and 16(10%) vs 59(20%) vs 53(18%) SS genotypes; with no statistical significance among groups of subjects.

For DAT1 VNTR, alleles 6, 9, 10, 11 when compared schizophrenia vs control we found 153(51%) vs 146(50%) 10/10 genotypes; 129(43%) vs 119(40%) 9/10 genotypes; 14(5%) vs 25(9%) 9/9 genotypes and 7(2%) vs 5(2%) other (9/6, 9/11, 10/6, 10/11) genotypes, with no statistical significance among groups of subjects.

For COMT, Val158Met polymorphism we found 87(28%) vs 78(26%) Met/Met genotypes; 140(46%) vs 158(53%) Val/Met genotypes; 79(26%) vs 63(21%) Val/Val genotypes, also with no statistical significance among groups of subjects.

Conclusions: Unlike some other studies, we found no statistical significance of investigated genetic polymorphism relationship with any psychiatric disorders for all of three studied genes.

Serum fibroblast growth factor-21 is associated with renal sinus fat increasement independently of total intraabdominal obesity

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Pathways through which obesity might cause renal disease are not well understood. Recent studies have associated ectopic lipid accumulation in the kidney with obesity-related renal disease. Human studies indicate that circulating levels of fibroblast growth factor-21 (FGF21) increased in obese individuals. FGF21 was found to closely associated with renal dysfunction in end-stage renal disease subjects. We hypothesised that renal sinus (RS) fat volume may be independently associated with increased level of FGF21.

The study included 110 subjects (60/50 F/M; age of 39.8±5.8). CT images were captured and RS fat accumulation was measured using the 3D-Doctor software. Both kidneys and RS fat were measured, and ratio left kidney sinus fat/ left kidney (LS/LK) and right kidney sinus fat/ right kidney (RS/RK) were calculated. Intraabdominal (IA) fat volume was measured at the level of renal hilus. FGF21 serum level was detected by ELISA assay.

Partial rank correlation was used to adjust the association between LS/LK, RS/RK and FGF21 after accounting for the IA fat volume. According on sex-specific 75th percentiles of FGF21 levels all measurements were divided into two groups and analysed by Mann-Whitney U test.

FGF21 correlated with both LS/LK and RS/RK ratios (r=0.50, p<0.001; and r=0.45, p<0.05). There were significant (p<0.05) increasement of both LS/LK (0.0075; 0.0019; 0.0165) and 0.0162 (0.0077; 0.0332)* and RS/RK (0.0027; 0.0003; 0.0051) and 0.0084 (0.0017; 0.0311)* ratios when data were divided according on sex-specific 75th percentiles (234.32 pg/ml) of FGF21.

Taken together, these results suggest that serum FGF21 level may be increased in individuals with reduced renal function because of the increased fat accumulation in the renal sinuses.

* Data are presented as median (25th, 75th percentiles)

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Nutrigenomics and epigenetics in obesity treatment strategy

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Recently it has been suggested that the existence of mammalian polyphenism in energy metabolism which might have implications for strategies to limit the obesity epidemic. Epigenetic marks, including CpG methylation, have been usually considered stable in somatic cells, although it has been observed that some environmental factors can cause variation or reversibility in the DNA methylation patterns in postnatal life. Epigenetic mechanisms might contribute to the worldwide increase in the prevalence of obesity. Thus, the pattern changes with ageing in a tissue-specific fashion, and an age-related increase in methylation is negatively associated with the expression of genes, suggesting that DNA methylation could be involved in increasing age dependent susceptibility to metabolic complications.

Epigenetic marks are tissue specific and include DNA methylation and histone modifications which mediate biological processes such as imprinting. As many imprinted genes are growth factors, or regulators of gene expression controlling growth, imprinting disorders often feature obesity as one of their clinical characteristics. The mechanisms by which nutritional challenges affect the risk of disease in later life are poorly understood. However, evidence indicates that the establishment of the epigenome can be affected by environmental factors during critical developmental periods. Possible disturbances of methylation may arise during fetal development due to lack of availability of dietary methyl donors. Potential interactions between the environment and epigenetic mechanisms mediating the expression of genes associated with increased BMI and adiposity, may also be possible as suggested for; the FTO locus is a DNA-demethylase enzyme, the MC4R gene which has reduced methylation following long-term exposure to a high fat diet, the PPARγ protein which interacts with histone acetyltransferases during adipogenesis and on the effect of diet on methylation of POMC and leptin. In the adult state, some examples of diet-induced epigenetic changes have also been reported. In this sense, folic acid (38,39) has been linked to DNA methylation in a dose-dependent manner, as reviewed by Kim et al. (60). Other dietary factors involved in DNA methylation

the gastrointestinal tract and colorectal cancer develop-ment are alcohol (39), vitamin B 6 (41,42), vitamin A and some minerals (43). Selenium deficiency also seems to be an important modifier of methyl metabolism in some tissues (44), while arsenite deprivation hypomethylates Caco-2 cells (45).Finally, not only micronutrients can induce epigenetics changes. A threefold increase in the expression of the human manganese superoxide dismutase (MnSOD) gene has been associated with a decreased CpG methylation comparing a vegetarian with an omnivore group (46). Other dietary factors such as fatty acids are likely to par-ticipate in epigenetic regulation by DNA methylation (47). For instance, butyrate induced demethylation of RARBeta2 in cancer cells (48). Tocopherols have been related to epi-genetic modifications of histones (49). Other compounds present in different plant foods such as the isofl-avone genistein (50), tea polyphenols (51) and garlic’s diallyl disulfide (52) are also able to regulate DNA Other factors that are able to alter DNA methylation patterns such as inflammation, oxidative stress and hypoxia are exacerbated in adipose tissue of obese subjects. In this regard, the relationship between obesity and the epigenetic regulation of gene expression has been recently reported by our group, showing that the successful response to a hypocaloric diet could be related to a lower methylation of the FTO promoter in PBMC cells. CpG hypomethylation of FTO promoter has recently been also associated with resistance to T2DM.

Our next major goal is to see whether different diet or therapy may modify this process, whether we can turn the disease switch “on” or “off” by supplementing diet, minimizing stress, or giving epigenetically relevant pro-drugs.

Epistatic interactions between VEGF, LSR and APOE on blood lipid traits

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Background: The Vascular Endothelial Growth Factor (VEGF) is a pivotal factor for angiogenesis and is involved in different diseases such as tumor growth and vascular disorders. We have previously identified 10 single nucleotide polymorphisms (SNPs) that explain up to 50% of VEGF variability2,3. We have also found associations between these SNPs and the levels of blood lipids. The levels of blood lipids, especially plasma cholesterol and triglycerides (TG) are risk factors for cardiovascular diseases (CVD). Apolipoproteins play an important role in the regulation of the processing and clearance of lipoproteins. Apolipoprotein E (ApoE) has been considered as an important risk factor for CVD. The lipolysis stimulated lipoprotein receptor (LSR) plays an important role in the removal of apoE-containing TG-rich lipoproteins during the postprandial phase.

Objectives: To investigate the role of potential epistasis of the GWAS published VEGF-related SNPs with LSR polymorphisms and with the established polymorphism of APOE on blood lipid levels in childhood and adulthood in healthy populations.

Design: Two populations from the STANISLAS Family Study (SFS) were used: unrelated healthy adults (n=432) and children (n=428, 18 years old).

Results: Epistatic interactions were identified in the models adjusted for age, gender, BMI and smoking between VEGF-related SNPs and LSR rs916447 for triglycerides in both children and adults, for apolipoprotein A1, CIII and E only in children, and for triglycerides, and apolipoprotein CIII only in adults. The interactions of VEGF SNPs with APOE were significant only in adults (triglycerides, cholesterol, apolipoprotein E).

Conclusions: These results indicating relationships between VEGF, APOE, and LSR reveal possible existence of complex molecular links between these three molecules for the regulation of lipid levels that is under investigation.
Decrease of insulin sensitivity, from obesity through pre-diabetes - importance of Matsuda index

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**Background:** Increased insulin resistance (IR) and decreased insulin sensitivity (IS) characterise obesity and early glycoregulation disorders: impaired fasting glucose (IFG) and impaired glucose tolerance (IGT). In this study we used Matsuda index of whole-body insulin sensitivity derived from the oral glucose tolerance test (OOGTT), which represents a composite of both hepatic and peripheral tissue sensitivity to insulin.

**Objectives:** To examine IS and IR in obese and pre-diabetic patients, and analyze thrombotic, inflammatory factors, microalbuminuria and HbA1C as the risk factors for developing atherosclerotic complications.

**Methods:** The study included 434 obese individuals (age over 45, body mass index (BMI) > 25 kg/m²) classified into three groups: I-obese (265); II-IFG (103); III-IGT (66). OOGTT was used to evaluate the extent of disorder. IDF classification was applied for diagnosing MS, IR was determined by homeostatic model assessment (HOMA IR). IS was determined by Matsuda index of whole-body insulin sensitivity.

**Results:** Blood pressure, HbA1C and HOMA IR values followed the progression of disorder. WC: (I-103.9±15.5, II-105.7±16.4 cm), HbA1C (I-5.6±0.9, II-6.0±0.8, III-6.0±0.5), Matsuda index (I-5.1±3.8, II-6.1±3.3, III-6.6±3.0), HOMA IR (I-6.0±1.7, II-6.1±1.8, III-6.6±2.0). Correlations: Statistical significance has been proved between groups: HOMA IR (p<0.01), Matsuda index (p<0.01), HbA1C (p<0.01), microalbuminuria (p<0.01). MS was found (I-61.9%, II-84.4%, III-80.5%). In advanced stage of the disease patients with MS had increased IR and decreased IS: HOMA IR (I-6.0±1.7, II-6.1±1.8, III-6.6±2.0), Matsuda index (I-5.1±3.5, II-6.6±2.7, III-2.6±1.7).

**Conclusion:** Decreased IS presented as decreased Matsuda index, exists in obesity and decreases more in pre-diabetes. Correlations of insulinemia with WC and HOMA IR confirm the importance of abdominal obesity in disorder etiopathogenesis. Highly important correlation of 0’, 30’ and 120’ glycemia with HbA1C, HOMA IR confirms the connection of increased IR and decreased IS with the progression of glycoregulation disorder. Our early results indicate beneficial effects of Mediterranean diet on obesity, IS, glycoregulation, lipid status, blood pressure and antioxidant protection in primary prevention of diabetes mellitus type 2 and atherosclerosis.


**Group B: Pharmacogenomics**

**The Role of ABCB1 (MDR1, P-glycoprotein) in the Disposition of Ciprofloxacin**

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**Background:** The international survey “Treat Infections in Neonates” (TINN) aimed to optimize the ciprofloxacin treatment of neonates and particularly the vulnerable group of premature neonates. In these patients, ciprofloxacin is an antibiotic prescribed off-label for the treatment...
of severe infections due to multi-resistant Gram negative bacteria. Its pharmacokinetics is highly variable and crucial for drug efficacy and its safety profile is unknown in neonates.

**Objective:** P-glycoprotein (P-gp), also known as multidrug resistance protein 1 (MDR1), is an ubiquitously efflux pump and its role in ciprofloxacin pharmacokinetics is still to be determined. In the present study, we investigated in human lymphocytes the impact of P-gp expression and polymorphism on ciprofloxacin intracellular concentrations and cell sensitivity. The influence of P-gp polymorphism was also studied on pharmacokinetic parameters calculated in neonates treated by ciprofloxacin.

**Design:** In vitro sensitivity of lymphoblastoid cell lines (LCLs) was evaluated by growth inhibition assay: 100 LCLs were incubated for 72 h with different concentrations of ciprofloxacin (10, 100, 300 and 600 µM). Intracellular concentrations of ciprofloxacin were analyzed by HPLC-UV. P-gp expression was evaluated by quantitative PCR and genetic variants (rs1045642, rs2032582, rs1128503, rs3842, rs1922262, rs2032583) were tested using a real-time TaqMan allelic discrimination method. Clearance (CL) and volume of distribution at steady state (Vss) calculated by population modeling were determined in 50 neonates included in the pharmacokinetic TINN study.

**Results:** The sensitivity of LCLs to ciprofloxacin (IC50) was dependent on the basal expression of P-gp: cells with low P-gp expression showed significant higher sensitivity to ciprofloxacin, compared to cells with higher gene expression (p= 0.038). This sensitivity of LCLs was associated with one variant of P-gp (rs1045642): cells carrying the genotype CC showed higher sensitivity to ciprofloxacin than those with wild-type genotype. The same tendency was observed with the variant rs2032583 (p = 0.052). Furthermore IC50 correlated with intracellular maximum concentration (Cmax) of ciprofloxacin (p = 0.013) and both P-gp variants described above impacted Cmax of ciprofloxacin.

In neonates, a different P-gp rs3842 impacted significantly ciprofloxacin clearance (p = 0.04) and volume of distribution at steady-state (p = 0.02).

**Conclusion:** P-gp polymorphism has a significant impact on intracellular concentrations of ciprofloxacin in vitro and neonatal clearance in vivo. Pharmacogenetics should be explored as a tool to optimize drug efficacy in neonatal treatment.

**Acknowledgements:** This work was funded through the TINN project.


**Insights into the biology and therapeutic implications of mediators and effectors of pro-metastatic focal adhesion signaling**

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Early stage progression of primary cancer to metastatic forms is initiated by excessive cancer cell locomotion in response to chemotactic signals received from cancer tissue microenvironment. In general, invasive motile cancer cells exhibit excessive finger-like plasma membrane protrusions that are stabilized by focal adhesions (FAs), a network of dynamic protein complexes formed near leading edges of cell protrusions to couple cell matrix receptors to the actin cytoskeleton. Synchronous assembly/disassembly of FAs at cell protrusions is essential for the generation of traction forces necessary for cancer cell locomotion and spread. In preclinical models, metastatic breast cancer cells express rapid disassembly rates of FA proteins at leading edges of cell protrusions. We identified members of the Rab-GTPase family, which are amplified in a subset of breast cancer subtypes, to contribute to accelerated FA disassembly via their recruitment to lysosomal and autophagosomal compartments for degradation, leading to a rapid FA turnover and excessive cell locomotion and invasion. Using genetic and pharmacological (small molecule inhibitors) approaches we established that inhibition of Rabs and autophagy prevented FA disassembly and efficiently inhibited cancer cell locomotion, as well as cancer progression to metastasis in animal models. The translational implications of these findings will be presented. **Supported by the Quebec Breast Cancer Foundation and the Canadian Institutes for Health Research.**

**Novel genetic variations in the promoter regions of solute carrier transporter genes within the Cape Admixed Population of South Africa**

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**Background:** Human solute carrier transporters (SLCs) are important determinants of organic cation and anion uptake from the circulation into the renal proximal tubules. These organic an/cations include clinical drugs such as metformin, lamivudine, cimetidine, cisplatin and the neurotoxin 1-methyl-4-phenyl-pyridinium. Considerable interindividual variation exists in drug responses and toxicity. Genetic polymorphisms in drug transporters are increasingly being recognized as a possible mechanism explaining this variation in drug disposition and response. Genetic variants in the basal promoter region, in particular, may have significant effects on the expression levels of drug transporter genes. This may result in changes in the activity levels of drugs that are substrates for these transporters and possible reduction in the associated clinical responses.
**Objective:** The aim of this study was to sequence the basal promoter region of SLC22A1, SLC22A3, SLC47A1, SLC47A2, SLC01B1 and SLC01B3 genes and to assess the extent of genetic variation in these genes within the Cape Admixed (CA) population.

**Materials and methods:** Ninety-six unrelated healthy subjects from the CA population were recruited for the study. The 5′-UTR regions of selected SLC genes were amplified using specifically designed PCR primers. The PCR products were sequenced using Sanger sequencing.

**Results:** Sequence analysis revealed 26 variations, of which 7 were novel mutations. Nineteen of the SNPs identified in this study were already reported and listed in the dbSNP database. The allele frequencies for these variants were compared to those observed in other admixed, African, Asian and Caucasian populations.

**Conclusion:** This study represents the first report of novel polymorphisms in selected SLC genes within the CA population. The data observed suggest that drugs which are substrates for these SLC transporters with novel genetic variants may exhibit different response profiles within this population.

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**Length variations of 3′-UTRs of ABC-drug transporters and consequences in microRNA regulation and pharmacotherapy resistances**

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**Background:** The 3′-UTR is an important regulatory motif of gene expression and the main binding site for microRNAs. In silico analysis of the 3′-UTRs of major ABC-drug transporters using common databases revealed length variations of these 3′-UTRs (alternative polyadenylation) and conflicting data to reported microRNA binding sites which are partially not located within 3′-UTRs as described. This is also true for commercially distributed vectors containing these 3′-UTRs linked to reporter gene constructs. The fact that the expression level of ABC transporters contributes to pharmacoresistances or insufficient drug responses necessitates a detailed analysis of the 3′-UTRs of drug transporters and their possible length variations.

**Objective:** The objective of this study is to identify the exact length and possible sequence variations of the 3′-UTRs of the major drug transporters ABCB1, ABCG2, ABCC1 and ABCC2 and to analyze the existence of different 3′-UTR lengths in various cell-lines as well as drug resistant cancer cells and their drug sensitive counterparts.

**Methods:** 3′-RACE and various standard PCR experiments were performed using cDNA of different human cell lines and primary human tissues. The abundance of 3′-UTR fragments was analyzed using quantitative RT-PCR.

**Results:** Five different ABCB1 3′-UTR length variants were identified. Previously reported microRNA binding sites were located only on the three longer fragments. Imatinib resistant leukemia cells expressed predominantly shorter 3′-UTRs, where miRNA binding sites are absent [1]. For ABCC2 also five different 3′-UTR length variants were identified. Reported microRNA binding sites for ABCC2 are completely absent on the shortest fragment. In silico analysis of ABCG2 and ABCC1 revealed six different 3′-UTR lengths for ABCG2 and eight for ABCC1. Experimental verification of the in silico analysis is currently in process.

**Conclusions:** Shortening of the 3′-UTRs of ABC-drug transporters cause loss of microRNA-dependent post-transcriptional mRNA silencing leading to elevated protein levels and elevated drug insensitivity or drug resistances, respectively.


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**CYP2D6 allele specific copy number analysis using TaqMan® SNP Genotyping Assays and digital PCR**

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The major drug metabolizing enzyme, CYP2D6, is encoded by a highly polymorphic gene. Over 100 star allele haplotypes are known, which can contain SNP, InDel, and copy number variants (CNVs) and which fall into 3 main functional categories (full, reduced, or none). The diploid star allele content is predictive of CYP2D6 drug metabolizer phenotype (ultrarapid, extensive, intermediate, or poor). We previously described a workflow whereby sample SNP genotype and CNV analysis results, generated using TaqMan® SNP assays and TaqMan® Copy Number assays, respectively, can be translated to star allele diplotypes using AlleleTyper™ software. However, for samples that carry CYP2D6 duplications and are heterozygous for key SNPs, the specific allele that is duplicated cannot always be identified. A phenotype can be assigned if all 3 alleles are of the same functional category, but if alleles are from different functional categories there may be 2 possible phenotypes. To address this issue, we developed a method to detect allele-specific copy number by digital PCR using the QuantStudio™ 3D Digital PCR System.
Potential role of single nucleotide polymorphisms of XRCC1, XRCC3, and RAD51 in predicting acute toxicity in rectal cancer patients treated with preoperative radiochemotherapy

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Background: Colorectal cancer is the third most common cancer in the western world and preoperative radiochemotherapy and total mesorectal excision are widely used for the treatment of locally advanced rectal cancer. Sometimes, neoadjuvant radiochemotherapy (N-RCT) is associated with high rates of complications reducing patient’s compliance during treatment. Genetic factors may influence tissues radiosensitivity and the development of side effects. Radiotherapy (RT) exerts its cytotoxic effects through the damage to cell structures, proteins, and DNA, then individual variation in the mechanisms of DNA repair and xenobiotic metabolism may modify the response of normal tissues. Some studies showed that polymorphisms of genes involved in the DNA repair pathways could influence cellular sensitivity to radiation and chemotherapeutic agents.

Objective: The aim of this study was to analyzed the association between polymorphisms of DNA repair genes and acute toxicity in rectal cancer patients treated with N-RCT.

Design: Sixty-seven patients were genotyped for the following SNPs: XRCC1 rs25487, XRCC3 rs1799794 and rs861539, RAD51 rs1801320 and GSTP1 rs1695.

Results: RAD51 correlated with acute severe gastrointestinal toxicity in heterozygosity (Aa) and homozygosity (AA) (P = 0.036). Grade ≥ 3 abdominal/pelvis pain toxicity was higher in the Aa group (P = 0.017) and in the Aa +AA group (P = 0.027) compared with homozygous (aa) patients. Acute skin toxicity of any grade occurred in 55.6% of the mutated patients versus 22.8% in the wildtype group (P = 0.04) for RAD51. XRCCI correlated with skin toxicity of any grade in the Aa +AA group (P = 0.03) and in the Aa group alone (P = 0.044). Grade ≥ 3 urinary frequency/urgency was significantly higher in patients with AA (P = 0.01), Aa (P = 0.022), and Aa +AA (P = 0.031) for XRCC3 compared with aa group.

Conclusions: Our study suggested that RAD51, XRCCI, and XRCC3 polymorphisms may be predictive factors for radiation-induced acute toxicity in rectal cancer patients treated with preoperative combined therapy.


Characterization of a novel single nucleotide polymorphism in thiopurine methyltransferase

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Background: Thiopurine drug induced toxicity is associated with defects in the thiopurine methyltransferase (TPMT) gene. TPMT is a polymeric enzyme, with most of the single nucleotide polymorphisms (SNPs) causing an amino acid change, which in most cases results in low enzymatic activity of the TPMT protein. The SNP for TPMT*3C, c.719A>G results in an amino acid substitution at position 240 from Tyr to Cys. Tyr240 is highly conserved and mutation at Tyr240 may alter the protein stability. Cellular studies show that the TPMT co-factor S-adenosylmethionine (SAM) could modulate TPMT activity. Although studies have been performed both with patient material and in cellular assays a limited number of studies have studied the direct effect of SAM on TPMT stability.

Objective: The objective was to characterize the novel patient allele c.719A>C together with the more common variant c.719A>G, resulting in amino acid shift from Tyr to Ser, p.Y240S and Tyr to Cys, p.Y260C respectively. In addition, the wild-type protein TPMT*1 was characterized and both variants were compared against TPMT*1.

Design: TPMT c.719A>C (p.Y240S) was identified using DNA sequencing and pyrosequencing. The enzymatic activity in red blood cells was determined. Recombinant TPMT c.719A>C protein, TPMT*3C and the wild type TPMT*1 were produced in E.coli, purified with standard methods and characterized using biophysical methods such as Circular Dichroism (CD) and Isothermal Titration Calorimetry (ITC). The recombinant enzymatic activity was determined using a fluorescence assay.

Results: The new TPMT SNP, c.719A>C (p.Y240S) have intermediate enzymatic activity in red blood cells. We evaluated the thermal stability and show that TPMT p.Y240S is less stable than both TPMT*1 and TPMT*3C. Furthermore, addition of SAM increases the stability for all protein variants, thus higher molar excess of SAM are needed in order to stabilize TPMT*3C and TPMT p.Y240S. In addition, SAM increases the long-term stability of both TPMT*1 and TPMT*3C. In agreement with thermal stability data, TPMT p.Y240S shows a remarkably decreased enzymatic effect compared to TPMT*1 and TPMT*3C.

Conclusions: Our studies gain further insight to the TPMT enzyme and the possible stabilizing effects of SAM. We show that both TPMT p.Y240S and TPMT*3C have lower enzymatic activity and are less stable compared to TPMT*1. All protein variants used in or study can be stabilized by the addition of SAM.
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Polymorphism within the RYR2 transporter as a potential predictor of statin associated myalgia/myopathy in Czech population

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Background: Statins are the most common drugs used in both primary and secondary cardiovascular prevention. Genetic variants might play a major role in determination of the adverse statin effects, like myalgia or myopathy.

Objectives: Gene for ryanodine receptor 2 gene (RYR2) belongs to the candidates with potential to influence risk of statin induced muscle problems (myalgia/myopathy). RYR2 codes for tetramer channel, which is major source of calcium required for muscle excitation-contraction and rs2819742 variant within this gene was associated with cerivastatin induced rhabdomyolysis. Design: RYR2 rs2819742 (G > A at chromosome 1, g.237990122) intronic polymorphism was successfully (CR 99.3%) analysed in heterogeneous group of 288 patients with statin induced myopathy (~ 90% on simvastin or atorvastatin, 10 mg or 20 mg per day) and 2, 492 healthy adult controls (none on statin treatment, CR 99.8%). Polymorphism was analysed using PCR-RFLP.

Results: Distribution of the individual RYR2 genotypes in Czech patients with statin induced myalgia/myopathy (GG = 30.0%, GA = 53.9%, AA = 16.1%) was slightly different from the control population (GG = 36.0%, GA = 48.1%, AA = 15.9%; P < 0.05). In comparison with GG homozygotes, carriers of at least one A allele were on higher risk of statin associated myalgia/myopathy development (OR [95%CI] = 1.31 [1.00 - 1.70]; P = 0.048).

Conclusions: Results of the pilot study suggest that the rs2819742 variant within the gene for RYR2 receptor could be associated with statin induced myalgia/myopathy in Czech patients on low doses of common statins. Supported by project MH CR - IN 00023001, IKEM.

Clinical barriers for the application of pharmacogenetics in the UK: statistical analysis of limitations of pharmacogenetic resources with a focus on warfarin and clopidogrel treatment and impact of the decreasing costs of pharmacogenetic tests

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Background and Hypothesis: A large amount of evidence is available to physicians in UK about pharmacogenetic tests for prescription of Warfarin and Clopidogrel. The resources available to support the cardiologists on how to and when to use pharmacogenetic tests need to be more accessible. The lack of clear guidance and high perceived costs of the pharmacogenetics tests act as barriers to adoption of testing before prescription. The principal aims of this study are:
1. To understand the opinion of cardiologists about the available resources that provide guidance on pharmacogenetic testing with respect to Warfarin and Clopidogrel, 2. To determine the limitations of the current resources, 3. To access the awareness about the decreasing cost of the pharmacogenetic tests.

Design: The survey methodology that has been designed for the purpose of this project includes a questionnaire (15-20 questions). This questionnaire will be used to record information about the knowledge gap, limitations of the resources and the decreasing costs of pharmacogenetic testing. As the study focuses on pharmacogenetics pertaining to warfarin and clopidogrel, the sample will mainly consist of cardiologists. The data generated will be analysed using non-parametric tests to identify statistically significant relations in the data. Main Findings and Project Impact: This project will make an important assessment about the impact of the support mechanisms available for the interpretation of pharmacogenetic tests for warfarin and clopidogrel and perceived costs associated with genetic testing. The results of the study will be used to make some inferences about the improvements that are needed in the interface between the cardiologists and pharmacogenetic testing.

References: Johansen Taber K, Dickinson B. Pharmacogenomic knowledge gaps and educational resource needs among physicians in selected specialties. PGPM. 2014;i145.
Correlation between CYPs 1A2, 2C9, 2C19, 2D6 and 3A4 hydroxylation phenotype and genotype in Ecuadorians

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Background: Genotype analysis of CYP450 enzymes is an essential, but not definitive to determine the interindividual variability observed for drug metabolism, as of the genotype-phenotype discordance observed. Therefore, the analysis of the CYP450 hydroxylation capacities should help to predict individual’s responses to the administration of more than 75% of prescribed drugs.

Objective: to explore the relationship between the main CYP450 genetic polymorphisms and the actual hydroxylation capacity (metabolic phenotype) in Ecuadorians.

Design: 139 healthy individuals were genotyped and phenotyped by using the CEIBA cocktail approach1. Plasma concentrations of the probe drugs and metabolites were quantified and metabolic ratios were calculated to determine each individual’s actual CYPs metabolic capacity. The genotype-phenotype relationship was evaluated by correlation analysis between ‘activity score’ and/or genotype, and MRlog.

Results: No adverse effects were reported and, according to the genotype analyses, no poor metabolizers were found for CYP2C9, whereas 0.752% and 3.10% of the subjects were classified as poor metabolizers for CYP2C19 and CYP2D6, respectively. Additionally, a prevalence of ultrarapid metabolizers (gUMs) of 15.79% for CYP2C19 and 5.43% for CYP2D6 was found. Metabolic ratios correlated with activity score for CYP2C9, CYP2C19 and CYP2D6 and with CYP1A2 genotype. Nevertheless, the frequency histograms and probit analysis showed no clear distinction among individuals when grouped by genotype or activity score, and all the individuals with lower drug metabolizing capacity carried zero active genes. However, some individuals carrying enhanced activity alleles showed lower drug metabolic capacity than individuals with reduced activity alleles.

Conclusions: This is the first study that simultaneously determined the genotypes, drug metabolizing phenotypes and analyses their correlation for the main CYP450 enzymes on Ecuadorians. A key role of the genetic polymorphisms is observed, though the actual metabolic capacity needs to be confirmed by a phenotyping approach.

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Interethnic CYP2D6, CYP2C9 and CYP2C19 variability across Native American populations and the relevancy of ancestry

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Background: The genetic polymorphisms of CYPs (CYP2D6, CYP2C9, and CYP2C19) are one of the major determinants of the interethnic variability in the drug response. Pharmacogenetics studies have been widely developed in Caucasians, but further studies are required in other populations, such as Latin-Americans (resulting of interethnic crosses among Amerindians, Europeans and Africans), and specifically in the understudied Native Americans.

Objectives: The CEIBA-RIBEF Network Consortium (European Ibero-American for Population Pharmacogenetics) aims to evaluate the CYP2D6, CYP2C9 and CYP2C19 genotype and phenotype in different Ibero-American populations.

Design: A total of 6060 individuals from Ibero-America were classified according to their self-reported ancestry in Natives (n=1395), Blacks (n=96), Caucasians (n=1824), Ashkenazi Jews (n=174) and Admixed Americans (n=2571). Moreover, Native-Americans were divided in three groups: North, Central- and South-Native Americans (from Mexico, Costa Rica and Peru, respectively). All subjects were studied for the most relevant CYP2D6, CYP2C9 and CYP2C19 allelic variants, and grouped into poor/ultrarapid metabolizers (gPMs and gUMs, respectively). Differences among groups were evaluated using χ2 test followed by the Marasculo procedure.

Results: Interethnic differences were detected among Native Americans and other ethnic groups: the frequency of CYP2C9*2 was lower in Native Americans (1.14%) than in other ethnic groups (7.96-16.63%; p<0.05), except for Black Americans. Moreover, the frequency of CYP2C19*17 was lower in Native Americans (1.59%) than in the rest (12.33-21.34%; p<0.05), and, consequently, CYP2C19 gUMs was less frequent among Native Americans (2.74%) compared with other ethnic groups (20.71-32.93%; p<0.05). Likewise, important differences within the Native Americans have been found: CYP2D6 gPMs were more frequent in Central-Native Americans (10.20%) than in the rest of Native Americans (0.00-0.31%; p<0.05), whereas CYP2D6 gUMs showed a higher frequency in North-Native Americans (9.52%) than in South- and Central Native Americans (0.41-3.57%; p<0.05).
Conclusions: For clozapine, there were indications that *1C increased the risk of adverse effects. For this reason, plasma concentration guided dose adjustment is recommended. This recommendation was incorporated in the decision support system of all Dutch pharmacies, general practices and hospitals and appears when clozapine is prescribed for patients labeled as *1C/*1C or *1C-heterozygote.

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Personalized cancer radiotherapy: a role for DNA repair SNPs as radiogenomic biomarkers?

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Background: Ionizing radiation has the ability to induce DNA damage and, therefore, to activate apoptotic cell death. This ability is the basis for cancer radiotherapy but normal cells may also be affected alongside with tumour cells. Since DNA repair mechanisms exist to counteract radiation-induced DNA damage, it is possible that variants in coding or regulatory regions of DNA repair genes may influence the efficacy and safety of radiotherapy and, ultimately, affect the clinical outcome of cancer patients submitted to such therapy.

Objective: Through systematic review of the clinical evidence available, we aimed to identify DNA repair SNPs that may correlate with the clinical outcome of cancer patients submitted to radiotherapy. Such SNPs have the potential to be used as radiogenomic biomarkers in personalized cancer therapy.

Design: The PUBLMED database was searched using the following MeSH terms: neoplasms AND Polymorphism, Single Nucleotide AND DNA repair AND radiotherapy. 104 articles, published up to March 1, 2016, were retrieved. On applying predefined inclusion/exclusion criteria, 79 articles were excluded. 25 articles were thus eligible for further data extraction.

Results: A high number of significant associations were observed between multiple clinical outcome endpoints (e.g. overall or disease-specific survival) and SNPs across different DNA repair pathways (direct damage reversal, base excision repair, nucleotide excision repair, homologous recombination), DNA damage response genes (e.g. ATM) and other genes with suspected DNA repair function (e.g. RECQL). The XRCC1 Gln399Arg substitution (rs25487) was the DNA repair SNP most frequently associated with response to radiotherapy, the variant allele being consistently associated with decreased survival. Associations between the XRCC2 Arg188His (rs3218536) variant allele and decreased survival and between the ERCC2 Lys751Gln (rs13181) variant allele and increased survival are also suggested by different studies. Despite less consistent, evidence also exists regarding the synonymous ERCC3 substitution Asn118Asn (rs16165).

Conclusions: DNA repair SNPs across different DNA repair pathways appear to modulate the individual clinical outcome in response to radiotherapy. Evidence is stronger for the XRCC1 Gln399Arg substitution (rs25487). Integration of such radiogenomic biomarkers into the clinical decision process may, in a near future, allow for the optimization of the therapeutic approach to several types of cancer, maximizing the benefit while minimizing the risk.

The most prevalent variant allele of CYP1A2 does not affect clozapine and olanzapine pharmacokinetics

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Background: The inactivation of clozapine and olanzapine is increased by smoking, which induces CYP1A2. The most prevalent allele of CYP1A2 in the Netherlands (*1F) has been reported to increase this induction compared to the second most frequent allele (*1A). An infrequent allele (*1C) results in diminished CYP1A2 activity.

Objective: To provide a national guideline on whether and how clozapine and olanzapine treatment should be adjusted in patients with CYP1A2 variant genotypes.

Design: Pharmacokinetics of clozapine and olanzapine did not differ between CYP1A2 *1A and *1F. This was also true in smokers. For clozapine, there were indications that *1C increased the risk of adverse effects. This reason, plasma concentration guided dose adjustment is recommended. This recommendation was incorporated in the decision support system of all Dutch pharmacies, general practices and hospitals and appears when clozapine is prescribed for patients labeled as *1C/*1C or *1C-heterozygote. For olanzapine no effect of *1C on pharmacokinetics or clinical effects was found.

Conclusions: No difference in pharmacokinetics of clozapine and olanzapine was observed for CYP1A2 *1A compared to CYP1A2 *1F. For clozapine, CYP1A2 *1C might increase the risk of adverse effects.
Acknowledgements: This work was funded by the Royal Dutch Pharmacists Association and through the Horizon2020 project ‘Ubiquitous Pharmacogenomics (U-PGx): Making actionable pharmacogenomic data and effective treatment optimization accessible to every European citizen’ (update and implementation in other European countries).

Reference: Earlier work:

Iatrogenically induced opioid dependence, pharmacogenetics and pain

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Aim of Investigation: Opioids have high therapeutic interest as analgesics but in a low percentage of patients, could associate aberrant drug-related behaviour. This is scarce studied in non-cancer chronic pain patients.

Methods: A cohort study was performed with outpatients (n=70) diagnosed with opioid iatrogenic dependence. They followed a structured and progressive opioid reduction dose program with conversion to buprenorphine or tramadol, along 6 months. Study was focused on analgesic efficacy, abstinence (Opioid Withdrawal Scale, OWS), adverse side effects, functional status and aberrant drug-related behaviour. Genotyping Single Nucleotide Polymorphism of the OPRM1 (rs1799971), OPRD1 (rs6985), COMT (rs4680), ABCB1 (rs1045642) and ARRB2 (rs609) genes was performed by real-time PCR. Ethical Committee approved the study. Statistical analysis was performed using R.3.2.0.

Results: A significant reduction of the total daily dose (TDD) converted to morphine, was achieved (267.25±163.72 vs. 115.03±100.14, p=0.033) without abstinence, presenting a moderate pain intensity. Quality of life tended to improve, as well as, the number of adverse reactions reported by the patients throughout the visits. Genotype 118-AA for OPRM1 gene required significantly lower TDD (codominant model, AA=90.83±15.36; AG=134.05±27.05; GG=308.40±15.36; p=0.037).

Conclusions: Our program showed effectiveness in reducing TDD with a good conversion to buprenorphine, significantly associated to 118-AA OPRM1 genotype.

Impact of ABC gene variants on plasma lipid traits and response to atorvastatin in Chilean individuals

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Background: Statins constitute the first line treatment to reduce high cholesterol levels. However, there is great interindividual variability in response to these drugs.

Objective: The goal of this study was to investigate the influence of four single nucleotide polymorphisms in genes of the ABC transporter family (ABCC2 rs717620, ABCG2 rs2231142, ABCBI rs1128503, and ABCBI rs3842) in the response to short-term low-dose atorvastatin medication in Chilean hypercholesterolemic patients.

Design: We included 127 Chilean hypercholesterolemic patients treated with 10 mg/day atorvastatin for one month. Lipid profile was determined before and after drug administration. Genotyping of the variants rs1128503, rs3842, rs717620 and rs2231142 was performed using TaqMan® Drug Metabolism Genotyping Assays.

Results: Following statin therapy, there was a reduction of TC, LDL-C and TG concentrations (p<0.05). Also, HDL-C levels increased (p<0.05). Minor allele frequencies for rs1128503, rs3842, rs717620, rs2231142 variants were 0.453, 0.154, 0.232 and 0.075, respectively. LDL-C response to atorvastatin was not associated with none of the studied polymorphisms (p>0.05). However, the rs3842 was associated with TG variability after atorvastatin medication in both men and women (p=0.0035), while rs717620 was associated with TG variability after atorvastatin medication in men only (p=0.0039).

Conclusion: This study indicates that LDL-C reduction following atorvastatin therapy is not influenced by the SNPs evaluated. However, the ABCBI rs3842 and ABCC2 rs717620 polymorphisms showed an association with TG concentration in response to atorvastatin medication in Chilean hypercholesterolemic subjects.

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Evaluation of epigenetic mechanisms in THP-1 cells treated with statins

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Background: Statins are HMG-CoA reductase inhibitors. Alongside enzymatic inhibition, statins induce intracellular activation of transcription factors, leading to an increase in low-density lipoprotein receptors (LDLRs) coupled to the plasma membrane of hepatocytes, enhancing clearance of plasma cholesterol. Several proteins with key functions, which are regulated by statins, are involved in this pathway. However, to date, there are no reports describing epigenetic mechanisms contributing to their regulation.

Objective: To evaluate the effect of DNA methylation, histone modification and miRNAs expression in the modulation of intracellular cholesterol metabolism genes in monocytes treated with statins.

Design: THP-1 cells in the absence and presence of 10 µM of atorvastatin or simvastatin were cultured. Gene expression of HMGCR, LDLR, SCAP, INSIG1, INSIG2, SREBF1, SREBF2, MBTPS1, KPNBI and MBTPS2 was evaluated by real-time PCR. DNA methylation in specific areas of promoter regions of the LDLR, SREBF2 and HMGCR genes was evaluated by sequencing after modification with sodium bisulfite. The modifications in H3 and H4 histones was determined by colorimetric assays and miR-29a, miR-29b, miR-33a, miR-33b, miR-300 and miR-454 expression was evaluated by real-time PCR.

Results: Both statins induced overexpression of LDLR, HMGCR, SREBF2 and INSIG1 genes, while simvastatin additionally overexpressed SCAP, MBTPS1 and MBTPS2. No changes in methylation status were observed in the promoter regions of LDLR, SREBF2 and HMGCR. Only atorvastatin induced an increase in H3K4 di- and trimethylation, H3K9 monomethylation and H3K36 di- and trimethylation. Both statins increased monomethylation of H3K36 and H3K79 di- and trimethylation, along with an increase in H3K14 and H3K9 acetylation and H3Ser28 phosphorylation. Simvastatin increased monomethylation of H4K20 and H4Ser1 phosphorylation. Both treatments were associated with H4K5 acetylation and only atorvastatin was associated with a decreased H4K20 trimethylation. MiR-29b and miR-454 showed increased expression after treatment with both statins, while only atorvastatin induced miR-33b overexpression.

Conclusions: Both statins investigated overexpressed genes involved in intracellular metabolism of cholesterol, probably due to greater transcriptional activity as a consequence of the modifications observed in histones H3 and H4 and by miRs modulation. Our results constitute the first report evaluating simultaneously three epigenetic mechanisms.

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Cytochrome P450 2C19 polymorphism does not contribute to in-stent restenosis in Chilean patients who underwent percutaneous coronary intervention receiving clopidogrel

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Background: Several data proved the significance of CYP2C19*2 polymorphism in the development of in-stent restenosis in vascular disease after endovascular treatment and stent implantation. In addition, it has been reported that single nucleotide variants within the CYP2C19 gene, the hepatic enzyme involved in clopidogrel biotransformation to its active metabolite, result in significant interindividual variations regarding therapeutic efficacy of the drug. Clopidogrel is a widely used antiplatelet drug for secondary prevention of atherosclerotic events in high risk patients, especially those undergoing percutaneous coronary interventions (PCI), who receive it in combination with aspirin as a dual antiplatelet therapy.

Objective: The aim of this study was to evaluate the impact of the loss of function variant CYP2C19*2 in the occurrence of in-stent restenosis in Chilean coronary artery disease patients who underwent PCI and received clopidogrel.

Design: A total of 144 patients were included. Patients with stenosis > 50% in the angioplasty site were defined as cases and those with < 50% as controls. Clinical and demographic variables were registered. CYP2C19*2 rs4244285 SNP was genotyped using TaqMan® Assays.

Results: The study included 68 cases (mean age 62.6 ± 9.9; 79.4% male) and 76 controls (mean age 64.9 ± 10; 71.1% male). Diabetes and bare metal stents (BMS) were observed more often in cases than controls (p=0.03 and p=0.002, respectively). Genotypic frequencies for the rs4244285 variant did not differ significantly between the groups (p=0.12). Nonetheless, the minor allele was observed in greater proportion in patients who did not develop in-stent restenosis (0.05 vs 0.12, p=0.04). There was no significant association between the loss of function rs4244285 variant and the occurrence of in-stent restenosis after PCI (OR= 0.4; 95% CI: 0.163 - 0.999; p=0.05).

Conclusions: We found no association between the CYP2C19*2 variant and coronary in-stent restenosis development in patients treated with clopidogrel. Our results should be interpreted with caution, since clinical and angiographic factors contribute to this condition, particularly diabetes, which is also associated with a low response to clopidogrel.

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Gender differences in genes polymorphisms of cytochrome P450 (CYP2C9, CYP4F2) and its impact on the phenindion dosage in patients with valvular atrial fribillation

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Background: Gender differences are important for personalized medicine.
Objective: To obtain the differences in phenindion dosing in men's and women's population, depending on polymorphisms of CYP2C9, CYP4F2.

Design: The study included 22 women (63.1 years), and 20 men (62.5), with valvular atrial fibrillation. The frequency of alleles and genotypes of CYP2C9 and CYP4F2 genes were performed by the method PCR-RFLP (restriction fragment length polymorphism). Statistical analysis was performed by using χ² Pearson and Student’s t-test.

Results: The target levels of INR (TLINR) (2.0-3.0) for women were achieved by the dose of phenindion 72,3±2,1 mg; for men - 88,5±6,7 mg (t=1,84, p>0.05). It was noticed out that the frequency of successful achieving TLINR differed due to the sex. So only 3 women (13.6%) haven't achieved TLINR (wINR-), on phenindion dose - 110±22,1 mg. 8 men (60%) haven't achieved the TLINR (mINR-) (x²=17,3, p<0.001), phenindion dose - 116,25±4,0 mg. 19 female patients who have achieved TLINR (wINR+) – on phenindion dose 66,3±3,5mg, 12 male patients (mINR+), the dose of phenindion was 70,0±4,7; (P>0.05). At the same time all patients were comparable for age, body mass index (30.1 for women and 30.7 for men). The following gene polymorphisms were found out in the group (wINR-): 1*1 CYP2C9 – 5 patients, 1*1 CYP2C9 – 3, CC CYP4F2 – 6, CT – 2 patients. In general the frequency of genes polymorphism 1*1 CYP2C9 (wild) in the group of (mINR-) was following: 1*1 CYP2C9 - 5 patients, 1*1 CYP2C9 - 3, CC CYP4F2 - 6, CT - 2 patients. The distri- bution of genes in the group (mINR+) was following: 1*1 1 CYP2C9 - 5 patients, 1*1 CYP2C9 – 3, CC CYP4F2 – (n=3), CC CYP4F2 – (n=3). The distribution of genes in the group (mINR-) was following: 1*1 CYP2C9 - 5 patients, 1*1 CYP2C9 – 3, CC CYP4F2 – 6, CT - 2 patients. In general the frequency of genes polymorphism 1*1 CYP2C9 (wild) in the group of (mINR-) was following: 1*1 CYP2C9 - 5 patients, 1*1 CYP2C9 – 3, CC CYP4F2 – 6, CT - 2 patients. In general the frequency of genes polymorphism 1*1 CYP2C9 (wild) in the group of (mINR-) was following: 1*1 CYP2C9 - 5 patients, 1*1 CYP2C9 – 3, CC CYP4F2 – 6, CT - 2 patients.

Conclusions: The male patients with WAF that didn't achieve TLINR on the adequate therapy of phenindion were found out for the three times often than women and in the men's group cytochrome P450 polymorphisms were presented more diverse.


The Effects of Glipizide and Acarbose on Obesity and Glucose Metabolism in 3T3-L1, AML12 cell lines and their co-cultures

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Background: The unipotent preadipocytes 3T3-L1, are ideal for studying the molecular events responsible for the conversion of preadipocytes into adipocytes since they can either remain as preadipocytes or undergo conversion to mature adipose tissue. Hepatocytes, are commonly used in gene regulation studies.

Objective: The aim of the study is to evaluate how The active substances of oral-anti-diabetic drugs such as glipizide and acarbose, effect the cells reproduction activities and how they change the expressions of FTO, CD68, NIBAN, and RAN genes which could change insulin signalization, also be effective in the adipogenetic process, have unknown functions and related to obesity in 3T3-L1 adipocytes, AML12 hepatocytes and adipocyte-hepatocyte co-cultures.

Design: Cell proliferation was examined with iCELLigence system. The time and amount of active substances of the oral-anti-diabetic drugs which were applied to cells were determined real-time according to IC50 value. FTO, CD68 NIBAN and RAN gene expressions were determined with qPCR.

Results: When single and multiple doses of glipizide and acarbose in co-culture’s were compared respectively, the 24 hour IC50 values were determined as 180 µM and 17 mg/ml in adipocytes, 72 µM and 23 mg/ml in hepatocytes, 41.5 µM and 5 mg/ml in co-culture cells. FTO gene expression was found to be decreased in the glipizide administered cells, except for co-cultured hepatocytes. CD68 gene expression was found to be decreased in the glipizide administered cells, except for co-cultured hepatocytes. As a response to glipizide application, NIBAN gene expression was found to increase in adipocytes, whereas decreased in the co-cultured adipocytes. As a result of glipizide application, while RAN gene expression in co-cultured hepatocyte was silenced in other study groups it was increased. In the acarbose applied cells FTO and CD68 gene expressions were decreased in all groups. In the acarbose applied cells while NIBAN gene expression was increased in adipocytes, it was found to increase in co-cultured adipocytes. In the acarbose applied cells RAN gene expression was found to increase in all groups.

Conclusion: When compared to single cultures, the low drug doses in co-cultures shows that the doses that are determined, as proper doses less harmful, by determining specially personalized dose can be used in treatment instead of using high doses. The decreasing effects of glipizide and acarbose on CD68 and FTO gene expressions may show the protective effect of drug on inflammation and obesity. In glipizide and acarbose applied insulin resistant mature adipocytes, NIBAN gene expression’s high levels may show antiapoptotic effect and as to RAN gene expression’s high levels may show it’s regulatory effect in glucose homeostasis.

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Modelling the Structural and Functional effects of nucleotide polymorphisms in the human NAT1 drug metabolising enzyme

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The human drug metabolising enzymes (DME), arylamine N-acetyltransferases (NAT1 and NAT2) determine the duration of action of amine-containing drugs by impacting the metabolic balance between the activation and detoxification of these compounds. Single nucleotide polymorphisms (SNPs) in the NATs have been implicated in both the inter-ethnic and inter-individual variation in the phenotypic treatment profiles of patients. We investigated the effect of several novel non-synonymous SNPs on the structure and function of the NAT1 enzyme by simulation modelling studies. Interestingly, these SNPs were not found in nor associated with the catalytic pocket of the enzyme. The in silico prediction and simulation analyses utilised tools such as GROMACS, mCMS, SIFT and POLYPHEN-2 to investigate the effects of the SNPs on the human NAT1 enzyme. For the purpose of the analysis we used the wild type NAT1 crystal structure (PDBID: 2JIA), containing the acetylated cysteine-68 residue complexed with coenzyme A. In addition, the substrate para-aminobenzoic acid (PAS) was also complexed to the structure. The NAT1 SNPs were identified in a South African population cohort of mixed ancestry. The simulation results showed that two of the investigated novel SNPs, resulting in changes at amino acids 231 (V>G) and 245 (N>R) respectively, affected the function of the NAT1 protein, whilst two others, at 262 (R>W) and 264 (E>K) gave contradictory results, and could not be interpreted. Three of the altered amino acids, 231G, 242I and 264K showed stabilisation energies between -0.58 and -5.09 kcal/mol which is indicative of protein destabilisation. In addition the simulation studies indicated that the amino acid substitutions at V231G and E264K, also occupied energy minima in support of a predictive protein destabilisation. NAT1 SNPs have generally not been validated in vitro, to our knowledge. We propose that the use of simulation studies could provide an appropriate alternative to validate and quantify unknown SNP effects on protein structure and function, particularly as a selective screening methodology. The observations from this study could inform a strategy for incorporating genotypic data (i.e., functional SNP alleles) with phenotypic information (slow or fast acetylators) to prescribe more effective treatment regimens.

Characterization of the single nucleotide polymorphism 497A>G in the drug metabolizing enzyme thiopurine methyltransferase

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Background: Thiopurine methyltransferase (TPMT) is a polymorphic enzyme that converts cytotoxic thiopurine drugs into both active and inactive metabolites. In Caucasians 10 % carries a single nucleotide polymorphisms (SNP) causing a less functional enzyme leading to an increased risk of severe adverse reactions during treatment with normal thiopurine doses. Today about 40 SNPs are known and named by the TPMT nomenclature committee (www.imb.liu.se/tpmtalleles).

Recently, we discovered a SNP in the drug metabolizing enzyme TPMT, in a young female patient with acute lymphoblastic leukemia. It had earlier been reported from the genomic ClinSeq Project but has not been further characterized.

Objective: The objective of this study was to characterize the TPMT SNP 497A>G, to describe the properties and function of the resulting protein in comparison to the wild type TPMT.

Design: In our study the TPMT 497A>G was characterized in context of enzyme activity in RBC and WBC, cDNA sequencing and heredity. We also produced recombinant human TPMT 497A>G in Escherichia Coli bacteria and investigated the stability and structural changes of the protein structure with biophysical methods (1).

Results: The SNP consists of a 497A>G mutation which cause an amino acid shift of Y166C. Our results shows that 497A>G is a heritable variant of TPMT that decrease the patient’s enzyme activity dramatically. The overall structure of the enzyme is normal but the amino acid shift causes a pronounced less stable enzyme.

Conclusions: We present a full characterization of the nonfunctional TPMT SNP 497A>G, a SNP that influence the metabolism of thiopurine drugs, widely used during treatment of childhood leukemia.

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