Abstracts“)

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Systems Medicine, Personalized Health and Therapy
in collaboration with ESPT

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Systems and health

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Background: We live in a time of rapidly changing environments. Independently as unique individuals, and collectively as families, communities and societies we must adapt to live in these environments. Survival is paramount. Yet, it is an inherent biological component of life to tend toward health. How do we highlight and support health when non-communicable, chronic diseases (NCDs) threaten the hearts, minds and bodies of individuals and governments alike? NCDs affect not only personal health, but also the public wellbeing and health economics. One cause for optimism is that the biological bedrock of many NCDs contains a common thread of inflammatory disregulation. A deeper and integrative understanding of the causes and conditions that lead to such disregulation can provide novel interventions that may successfully target diverse NCDs. At the individual and family levels, the co-created development of the human immune, nervous and microbiome systems provide a scientific framework on which to prototype solutions. We also live in a time where data generation has become significantly cheaper than data analysis/utilization. The trend is general, especially with unstructured data. Structuring data adequately is unaffordable. This is an unprecedented trend – one that life scientists have never faced before. In addition, decision-making – using such data as input – is increasingly interwoven with the data itself. Despite the existence of an unprecedented amount of data, there is actually not enough to be purely data-driven in most cases and interdependency of data matters (a lot).

Objective: To explore the use of information and communication technologies to understand and discovering relationships pertaining to health and identifying critical points of intervention.

Design: Two examples, one focused on largely unstructured and combined information and another focused on detailed molecular descriptions of gut-microbe interactions were developed dynamically combined information and another focused on detailed molecular models and the same underlying informatics infrastructure.

Results: Using USA data for obesity it was shown that friendship networks are important in building weight perception, setting weight goals, and measuring social marginalization among adolescents and young adults. In gut infectious diseases structural models were generated and calibrated using literature with model fitting to the experimental data supporting in silico experiments to generate model-derived predictions for validation via wet experimental testing iteratively with the model to generate novel hypotheses.

Conclusions: Because of the relational nature, coevolution and connectedness of human habitats, institutions, activities, health and food systems they represent a central concept for public policy/health and individual health. Use of advanced information and communication technologies for studying, designing, anticipating or monitoring intervention effects provides new opportunities for sustainable health and health systems.

Acknowledgements: Deep gratitude for co-creation with Janina Fariñas, Christopher Barrett, Madhav Marathe, Achla Marathe and Josep-Bassaganya Riera over the years and for permission to talk about some of their results and experiences.

Application of Human Biochemical networks for Systems Medicine

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Metabolism plays a central role in many human diseases, including diabetes, obesity and cardiovascular diseases. Shifts in metabolism and metabolite levels are often involved in human disease, either as cause or as consequence of pathogenic changes, and therefore offer great potential for diagnosis and elucidation of disease processes. Metabolic reconstructions describe the biological knowledge about a target organism in a structured manner and permit the conversion into a mathematical format and subsequent computation of physiological properties. As such, they facilitate the investigation of the mechanisms underlying genotype–phenotype relationships and indeed, inheritable metabolic diseases.

Here, I will present the most comprehensive metabolic reconstruction of human metabolism, which has been recently assembled in a community effort, and some promising biomedical applications. In particular, I will illustrate how this reconstruction and derived cell type specific models can be used to further our understanding of network wide, metabolic effects of single enzyme defects that are often associated with inheritable metabolic diseases. Using constraint-based analysis, we were able to predict novel biomarkers and to investigate systemic, metabolic effects of these rare metabolic diseases.

I will illustrate that the human metabolic reconstruction provides a deep insight into complex human metabolic phenotypes and disease states and represents a fundamental tool for the study of the systems biology of human metabolism.

‘SYSCILIA’-from systems biology to systems medicine of cilia dysfunction in human genetic disease

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Primary cilia are basically cellular signaling hubs, harboring amongst others the noncanonical Wnt and Hedgehog signaling systems. Their disruption upon genetic mutation leads to striking developmental defects and a plethora of disease phenotypes, the ciliopathies. Some ciliopathy-associated proteins were found to be physically or functionally associated in several distinct groupings, with limited connections to other crucial biological processes. Early proteomics studies have also suggested a discrete repertoire of about 1000 proteins within the organelle (i.e. <5% of the proteome) that were still in need of organisation into pathways and networks. Small, relatively isolated systems are often targeted by systems biology approaches under the assumption that a limited set of molecules and interactions will be more tractable for modelling systems. Cilia are thus ideal organelles for systems biology as they can be regarded as semi-closed systems, being both largely spatially and biologically separated from many other cellular structures and processes.

The EU-FP7 funded global ‘SYSCILLA’ consortium focuses on the classic systems biology activities of data generation, integration, assay development, model building and model refinement. We have identified and mapped the core of over 200 ciliary and ciliopathy-associated proteins within the ciliary proteome into an integrated network that covers most of the ciliary space. By overlaying several high content datasets (siRNA screening data, genetic variation) and scrutinizing the associations in a systems-wide manner, we have generated models of cilium (dys-)function that support further diagnostic and therapeutic developments.

BioIntelligence & the Industrial Challenges for Systems Biology

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The purpose of the BioIntelligence Program is to develop an integrated digital environment for the discovery and development of new biological entities and products (from molecules to biological pathways, cells, organs, including regulatory aspects) for life sciences industries and research institutes, and in particular for pharmaceuticals, cosmetics, and agrochemicals.

This global collaborative environment for multidiscipline scientific innovation is aimed at:
- Proposing a unified platform for exploring and analyzing biological information (which is intrinsically heterogeneous and extremely diverse), and for formulating scientific hypotheses to be tested in the lab;
- Building in silico models supported by this bioknowledge, that can be numerically simulated and confronted to experimental data;
- Managing all discovery and development activities of those industries by relying on the foundations necessary to all involved multidiscipline R&D teams (collaboration, industrial processes coverage and certification).

The applications of this new platform will be presented, and will be demonstrated on a particular use case in a project of drug discovery in oncology.

SESSION II–FROM SYSTEMS MEDICINE TO SYSTEMS PHARMACOLOGY

The dawn of systems pharmacology and genome medicine

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Biologically active chemical compounds produce complex molecular responses already at the cellular level. The majority of compounds bind to several proteins: proteins of the cognate target class, those for which the compound was designed, but often also proteins bearing completely different folds. These proteins affect different pathways and cellular processes. While we usually monitor the net outcome of all these interactions in terms of selected biological readouts we are mostly oblivious of the intricacies that occur at the molecular level.

We have investigated the mechanism of action of several compounds in clinical use against cancer. We used a number of approaches in parallel: 1) chemical proteomics (affinity purifications with drug matrices/mass spectrometry), 2) chemical genetics (random mutagenesis of genome of near-haploid CML cells), 3) functional proteomics (affinity purification/mass spectrometry), 4) transcriptional profiling, 5) phosphoproteomics, 6) computational network analysis and modeling, and 7) validation by focused gene inactivation (RNAi and genome editing). We seek a detailed picture of the actual molecular events and requirements of the drugs under investigation. Using this integrated approach we have identified: 1) new targets for known drugs, 2) previously unknown mechanisms of drug resistance, 3) “effector” genes for the compounds (genes required for the drug to exert its action), 4) mechanisms of synergy between compounds and in a few cases 5) new medical use of existing drugs. This “systems-level” characterization of chemical entities should help understanding the biology of drug action better and allow the development of improved drugs. It should also help the community rationalizing patient stratification, thus increasing the efficacy of clinical trials and reducing unwanted side effects, but also contribute to the employment of mechanism-based combination therapy with existing drugs.

Pharmacogenomics and Alzheimer treatments

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The Phase III TOMMORROW trial is recruiting for the delay of onset of Mild Cognitive Impairment due to Alzheimer disease [MCI-AD] using a Biomarker Risk Assignment Algorithm (BRAA) that incorporates genotypic information at TOMM40 rs10525623, APOE and age at entry into the trial. [1] The algorithm is an enrichment tool to assign cognitively normal individuals between the ages of 65-83 with
respect to whether each individual has a high or low risk of starting symptoms and signs of MCI-AD in the next five years. Thus the trial is segmented for risk assignment according to the highly informative age of onset distributions based on TOMM40'S23 genotypes. This is a rapid translation from gene discovery to a Phase III clinical trial.

This has led to the development of a new genomic strategy for using highly variable structural variations (SV) found in introns and other DNA regions with a paucity of exons – the huge proportion of the genome responsible for much of the “missing heritability.” [2] This strategy begins with the creation of an SVdb. There is then a co-localization step using candidate genes to identify those candidates that are located adjacent to highly polymorphic SVs in the same linkage disequilibrium (LD) region. This highly polymorphic genetic marker in LD with a candidate gene can then be examined by phylogenetic mapping to identify the specific evolutionary clades associated with disease expression. This provides a starting point for an analysis of any candidate gene and the genetic power to generate highly statistically significant associations without genome wide association studies using millions of SNPs. The many genes labeled as candidates from GWAS experiments can also be tested for genetic association with a SV in LD to remove the current limitations and select likely biological candidates for further investigation. This strategy grew out of the TOMM40’S23 association discovery and has now been applied successfully to other complex diseases. With respect to the pharmacogenomics of AD, this strategy using focused phylogenetic analysis can be rapidly applied to investigator’s favorite gene as an independent confirmation of GWAS results. This generates association in specific clades that contain the highly informative SV and can strengthen the rationale for further translation investment.

References

2- Roses, A.D. et al., Solving “missing heritability” of human complex diseases: identifying candidate genes located in “junk” DNA. Molecular Psychiatry, in press

Genomics approaches to guide Multiple Sclerosis therapy

I. Grossman, Teva, Tel Aviv, Israel

Integrating Big Data into Translational Sciences at IPSEN

P. P. Denèfle,
Assoc. Professor, Cedep INSEAD, Sr Partner & Cofounder, MedBiomiX partners

Pharmaceutical industries are desperately seeking ways to cut costs, improve efficiency and provide better care. In parallel, digital data are exploding; from Clinical study to individual genomic data and many early clinical trials are now fully embracing the potential to tailor the therapeutic regimen to the individual disease molecular and genomic characteristics. Ten years after the first Human genomic DNA has been sequenced, the acceleration of human genome sequencing can enable to predict that most patients will be sequenced in the next ten years. However, a lot more potential is to be expected if one starts compiling other data dimensions, such as medical care systems data, imaging data legacy as well as other web-based public data. Indeed, between digitalized health records, imaging systems, healthcare claims, public health reports and the emerging market of telemedicine and computer-aided medical decision making, the healthcare industry is full of data that’s just waiting to be dissected.

25 September 2014 – Afternoon

SESSION III–UNDERSTANDING CANCER THROUGH SYSTEMS MEDICINE

Drug resistance in cancer cell populations: Genetic or epigenetic phenomenon? Mathematical and biological assessment

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Considering cancer as an evolutionary disease, we aim at understanding the means by which cancer cell populations develop resistance mechanisms to drug therapies. Rather than focusing on molecular mechanisms such as overexpression of intracellular drug processing enzymes or ABC transporters that are responsible for resistance at the individual cell level, we propose to introduce functional phenotypes (that may be experimentally identified and controlled in cell cultures, according to the drug and to the cell line at stake) of resistance structuring cancer cell populations. The models proposed may be considered as generalising evolutionary game theoretical models, in as much as the phenotypes are called strategies in other contexts, except that ours are not indexed by such and such a cell subpopulation (e.g., sensitive vs. resistant or multi drug resistant) but continuous and structuring the whole cell population in extent of expression (e.g., between 0 and 1) of the resistant phenotypes.
Drug-induced drug resistance, the question we are tackling from a theoretical and experimental point of view, may be due to biological mechanisms of different natures, mere local regulation, epigenetic modifications (reversible or not) or genetic mutations (irreversible), according to the extent to which the genome of the cells in the population is affected. In this respect, the models we develop are more likely to be biologically corresponding to epigenetic modifications, although eventual induction of emergent resistant cell clones due to mutations under drug pressure is not to be excluded. From the biologist’s point of view, we study phenotypically heterogeneous, but genetically homogeneous, cancer cell populations in cell cultures under drug pressure. According to the cell populations at stake and the exerted drug pressure, is drug resistance a permanently acquired phenotypic trait or is it reversible? Can it be avoided or overcome by rationally (model-guided) designed combinations of drugs (to be optimised)? These are some of the questions, rising from biological observations, that we try to answer in a collaboration between a team of mathematicians and another one of biologists, both dealing with cancer and Darwinian evolution of cell populations.

References


Cancer stem cells regulatory circuit is deciphered through an interactome–regulome–transcriptome integrative approach.

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Background: Tumor-initiating cells have been characterized in multiple cancers, including breast cancer (BC). So-called cancer stem cells (CSCs) present key stem cell properties including self-renewal (which drives tumorigenesis) and differentiation. They show resistance to conventional cancer therapies, and are thought to be the seed for the distant metastasis responsible for poor clinical outcomes. Unraveling deregulated mechanisms that are sustaining self-renewal and differentiation specific to CSCs is an essential step for the discovery of new research avenues and treatment in BC.

Objective: We aim to identify regulatory circuits driving breast CSC biology using a systems biology approach. The discovery of new druggable targets specific to the CSC population would allow refining personalized treatments in BC. These modules include information on regulation (transcription factors) as well as post-regulation (miRNA).

Design: After a functional whole genome screen of a miRNA library, we identified miR-600 as a modulator of CSC self-renewal and differentiation. We measured gene expression in CSCs after miR-600 overexpression and knock-down. We then used a two-step integrated approach based on the Interactome-Transcriptome Integration (ITI) algorithm4 in order to identify genetic modules post-regulated by miR-600. First, we identified modules with significantly deregulated expression in the human interactome (protein-protein interactions). Second, we identified pathways regulated by miR-600 in CSCs, using ITI and a regulation map based on TRANSPATH data (Protein-DNA interactions).

Results: By crossing the results of this two-step integrated approach, we established a list of deregulated modules in CSCs. We found 10 miR-600-regulated network modules driving CSC differentiation. Several transcription factors (RBMS, CEPPA, NMUR2), suppressors (EGR1, TP53), and genes known to regulate stem cell pathways (WNK1, a regulator of Wnt signaling) were identified.

Conclusions: Integrating gene expression data and network information (including physical interactions and regulatory relationships) allows the identification of biological pathways involved in CSC biology. These findings constitute a basis for anti-CSC drug target discovery in BC.

Acknowledgements: Support to this project is coming from the Institut National du Cancer grant Number INCA_5911 to CG.


Serum HER-2 determinations for personalized care of breast cancer patients during treatment with Herceptin.

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Background: Serum HER2 (S-HER2) was approved in 2003 by the US Food and Drug Administration (FDA) for monitoring trastuzumab treatment in tissue HER2 positive breast cancer patients. Information of the value of S-HER2 is scarce.

Objective: We hypothesized that S-HER2 would reflect the clinical effect of trastuzumab.

Design: We followed 68 patients eligible for trastuzumab treatment for up to 6 years or until death. S-HER2 was measured on an ADVIA Centaur System and S-trastuzumab was measured by an in-house developed fluorescent enzyme immunoassay system on the ImmunoCap 100. We also monitored 1,348 breast cancer patients to detect metastatic disease.

Results: A decrease in S-HER2 of >20% was correlated to no progression in the disease in 20 out of 21 clinical courses (p<0.0001). An increase in S-HER2 of >20% was correlated to progression in the disease in 40
out of 44 clinical courses (p<0.0001). Patients with no recurrence after trastuzumab treatment (n=18) had a median S-HER2 concentration of 10.5 μg/L, whereas patients alive with recurrence (n=013) had a median S-HER2 of 232.4 μg/L at latest measurement before death (p=0.0001) compared to patients without recurrence. In two patients with S-HER2 values above 1000 μg/L the concentrations of S-trastuzumab were measured below the target trough concentration in serum of 10 mg/L.

The sensitivity, specificity, positive and negative predictive values for detecting metastatic disease in tissue HER2-positive patients with values above 15 μg/L were 69% (95 CI 53-80%), 71% (62-78), 47% (35-59), and 86% (77-91), respectively. Combining the cutoff value of 15 μg/L with delta value of >100% increase from individual baseline after primary therapy, or increasing the cutoff to 32 μg/L raises the specificity to 96%, but lowers the sensitivity to 50 and 47%, respectively.

Conclusions: Decreasing values of S-HER2 predicts response to treatment whereas increasing levels predict resistance. S-HER2 above 1000 μg/L warns that standard doses of trastuzumab may be insufficient as reflected by low concentrations of S-trastuzumab.

Monitoring tissue HER2-positive breast cancer patients with serum HER-2 has a sufficient sensitivity to detect metastatic recurrence, while its use in monitoring of tissue HER2-negative patients is unsatisfactory.

Depression/cancer interaction and relevant genes

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In the interaction between cancer and depression several mechanisms for this association have been suggested including the increase of health risk behaviors and the activation of chronic stress among depressed patients.

Furthermore, chronic inflammation and oxidative stress have been implicated in the pathophysiology of Major Depressive Disorder (MDD), as well as in cancer development.

Moreover, major depressive disorder (MDD) is a highly prevalent disorder, which has been associated with an abnormal response of the hypothalomas-pituitary-adrenal (HPA) axis. It has been suggested that, in healthy individuals, central homeostatic buffering mechanisms regulating critical systems as the control of oxidation, hypoxia, and inflammation as well many others may become deregulated in untreated MDD leading to an increased risk of cancer development or a more aggressive cancer behavior. Cancer is a complex disease and the mechanisms of carcinogenesis are still controversial and needing more research to be totally clarified.

Since the proposal of the Hallmarks’ of cancer, it becomes clear that those are under the influence of central biological systems and sharing common pathophysiological mechanisms with depression.

In this review we discuss recent evidence from genetic polymorphism studies that are shedding more light to the interaction between depression and the different tumor models.

Towards Liquid Biopsies in Cancer and other Human Diseases

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DNA fragments from remote sites can be found in the peripheral circulation, typically at abundances of around 100 molecules of any genome sequence per cc of plasma. The fragments can arise by apoptotic events in diseased cells. These very low levels of analyte can be detected by the new ultraseek application of DNA mass spectrometry. But the amounts of material are too low to quantify accurately. In a typical disease situation there are multiple sequences of interest, and thus only a very sensitive technique which can detect multiple loci at once is suitable. A number of studies showing the applicability of the ultraseek detection method for liquid biopsies of cancer patients will be described. Here one focuses on somatic mutations that are present in tumor cells but not in normal cells. With ultraseek there is excellent concordances between somatic mutations found in liquid biopsies and conventional tissue biopsies. Apparently the discordant results seen in earlier studies were due to lack of sensitivity of the methods employed. It can be anticipated that in other disease situations DNA fragments from remote sites affected by the disease will also be clinically useful, but here, since no somatic mutations are expected, one will have to use epigenetic DNA markers instead. Phenotypes caused by gene amplification will probably also be detectable but gene deletion is unlikely to be distinguishable from false negatives at the very low numbers of DNA molecules seen in plasma.

ESPT NETWORK ON PHARMACOCENTOMIC LABORATORIES (closed meeting)


SESSION IV–UNDERSTANDING BRAIN HEALTH AND DISORDERS THROUGH SYSTEMS BIOLOGY

Brain and endobiotic – xenobiotic interactions

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Psychotropic drug metabolizing enzymes, including cytochrome P450s, are present in the brain where they not only could contribute to local drug metabolism but also affect local biochemical homeostasis. CYPs, especially CYP2D6 and CYP2C19, are suggested to be involved in the transformation and metabolism of many endogenous substances including neurotransmitters and neurosteroids. Liver and brain levels of CYPs are highly dependent on genetic polymorphism which causes interindividual differences in drug levels and response. However, interindividual differences in local brain expression of these enzymes might also explain the reported associations between genetic polymorphism of e.g. CYP2D6 and CYP2C19 and personality traits, affective behaviour, and vulnerability to neuropsychiatric disorders For example, overexpression of CYP2C19 in mouse brain during fetal lead to complete callosal agenesis and a severely underdeveloped hippocampus. Mice with higher fetal CYP2C19 expression show a behavioral phenotype in adult life, with increased stress sensitivity and increased anxiety-like behavior. Stressful life events and stress sensitivity are major risk factors for psychiatric disease making this model highly interesting for investigating systems that are involved in regulating the stress response. The CYP2C19 transgenic mice furthermore...
showed a hippocampal phenotype as adults, with a smaller and more stress sensitive hippocampal formation that furthermore contained a drastically reduced number of immature (double-cortin positive) neurons. The maturation and formation of new neurons within the hippocampus has been shown to be critical for normal hippocampal function and the disturbances seen in the mouse model could be the explanation for the displayed smaller hippocampus. In the lecture the endobiotic interactions with the brain CYPs will be reviewed and discussed in relation to human brain diseases.

Genetics of human memory: from gene hunting to drug discovery

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Memory is a fascinating capability that allows us to store and recall events that uniquely define our lives. Memory also allows us to learn from others – a prerequisite for the development and transmission of human cultures. The relevance of memory becomes evident in conditions where memory functions are disturbed. Here I argue that the development and combination of high-throughput human genetic methods, of elaborated statistical approaches and of sophisticated methods to quantify memory at the neural systems level will facilitate the identification of novel memory-related genes in humans. Ultimately, a crosstalk between behavioral genetic studies and investigation of causality by molecular genetic studies will pave the way towards the identification of biologically important molecules and the development of novel drugs.

Human induced pluripotent stem cells for modeling Parkinson's disease: a Systems Biology approach

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Background: Understanding the molecular basis of neurodegenerative diseases has long been hampered by limited access to live human neurons. The advent of human induced pluripotent stem cells (hiPS) derived by reprogramming of patient’s somatic cells offers an unprecedented opportunity for disease modeling. hiPS are characterized by their potential to self-renew and can be directed to differentiate into specialized cell types, including neuronal progenitors and post mitotic neurons. Therefore they constitute a biological substrate that allows identification of disease-related pathways and molecular targets while also being appropriate for high-throughput cell-based studies.

Objective and design: To generate a cellular model for a genetic form of Parkinson’s disease (PD) by reprogramming patient-specific dermal fibroblasts and directing them to differentiate into dopamine neurons.

Results: We have recently generated hiPS lines from healthy human individuals and patients with a rare familial form of PD, harboring an autosomal dominant A53T mutation in α-synuclein (A53T-αSyn). Formation of αSyn aggregates is the hallmark for A53T-PD, but also for other inherited or sporadic forms of PD. By directed differentiation of hiPS from A53T-PD patients and age-matched unaffected individuals we have established cultures of human neuronal precursors and electrophysiologically active midbrain dopamine neurons. Moreover, we have used these cells as a relevant substrate to investigate factors controlling neurogenic commitment and differentiation on one hand, and PD-related pathological features on the other, using a Systems Biology approach. To this end, we have performed whole transcriptome analysis of A53T-PD-derived iPSCs as well as A53T-PD-derived neuronal precursors and midbrain dopamine neurons vis-à-vis cells from unaffected individuals, using new generation sequencing technologies. We have thus obtained a set of differentially expressed coding and non-coding RNAs that are being validated.

Conclusions: We anticipate that our strategy should yield new factors controlling the differentiation of human neurons and will guide us towards identification of new PD biomarkers and PD-related molecular targets that may evolve into novel therapeutics.

Acknowledgements: This project is supported by the Hellenic General Secretariat for Research and Technology Grants SYNERGASIA-Noiseplus-09SYN-21–969 and ARISTEIA-ParkinsonTransMed-2272

Screening of compounds for the prevention of tau-induced effects in Drosophila visual system contributing to independent and synergistic effects of the MAPT and SNCA genes in Parkinson's disease

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Background and Objectives: Interaction of α-synuclein and protein tau has been proposed as a possible mechanism in Parkinson’s disease (PD). The utility of the Drosophila system in testing candidate compounds against tau-dependent neuronal dysfunction has been determined.

Methods: An inversion polymorphism of the microtubule-associated protein tau (MAPT) gene defines two haplotypes, H1 and H2. We set out to determine whether a) H1 haplotype, b) SNPs included in H1, c) subhaplotypes within H1 defined by these SNPs, and d) the synergistic interaction between the MAPT H1H1 genotype and the SNP rs356219 from the 3′ region of SNCA (α-synuclein gene), are associated with PD, in patients and control subjects of Greek and Italian origin. We utilized transgenic fly strains carrying human tau genes expressed under the UAS (upstream activator sequence)/Gal4 system to investigate the ability of candidate compounds to inhibit tau-mediated degeneration of photoreceptor neurons.

Results: Overall the frequency of the H1H1 genotype was 63.35% in PD cases and 56.6% in controls (OR: 1.33, 95% CI: 0.98–1.79). There was no significant difference between cases and control subjects in the overall frequency distribution of H1 SNPs, H1 subhaplotypes, SNCA rs356219 GG marker and its combination with MAPT H1H1 genotype.
Pharmacogenetic of antidepressants and its implication for suicide

E.M. Peñas-Lledó and A. Llerena

1,2,3-dithiazoles attenuated the disruption of the regular array of ommatidia in Drosophila eye, but this result could not be replicated.

**Conclusions:** Our data show strong evidence of an association between the H1H1 genotype and PD. Findings from the present study might constitute a robust basis for the design of new compounds with improved activity in repressing transgenic tau in a Drosophila model.


Pharmacogenetic of antidepressants and its implication for suicide

E.M. Peñas-Lledó and A. Llerena

A higher frequency of CYP2D6 active genes was found among patients with eating disorders (EDs) vs. healthy controls. Moreover, a higher frequency of UMs CYP2D6 Ultrarapid Metabolizers (UMs, defined as subjects carrying more than two active alleles) was found among individuals who committed suicide vs. those who died from natural causes. Later we showed that CYP2D6 UMs among EDs have a greater risk of engaging in suicidal behaviour. To date suicide completion, lifetime history of suicide attempts, suicidal risk, and the severity of the objective circumstances related to the suicide attempt have been found to be more likely in UMs. One explanation for this relationship could be treatment failure with antidepressant drugs metabolized by CYP2D6. In support of this, a greater frequency of UMs was observed among patients with mood disorders that did not respond to antidepressant treatment with CYP2D6 substrates. Moreover, several studies have found poor response to antidepressant drugs or early dropout from monotherapy treatment with CYP2D6 antidepressant substrates in UMs. Therefore the effect of CYP2D6 on suicidal behaviour can be due to antidepressants (CYP2D6 substrates) therapeutic failure, but also to differences in the CNS regulation, or to both of them.

26 September 2014 – Morning

SESSION V–APPLYING OMICS TO DRUG DISCOVERY AND RESPONS

Specific software and drug discovery

A. Bril, Suresnes, France

**Advanced concepts in drug bioproduction from microorganisms**

F Képès, T Lepage

1,2,3-dithiazoles attenuated the disruption of the regular array of ommatidia in Drosophila eye, but this result could not be replicated.

**Conclusions:** Our data show strong evidence of an association between the H1H1 genotype and PD. Findings from the present study might constitute a robust basis for the design of new compounds with improved activity in repressing transgenic tau in a Drosophila model.


Genes based knowledge for the development of new therapies

N. Levy, Marseille, France

**Fast gene expression-based selection of putative membrane-associated cancer antigens**

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**Background:** Monoclonal antibodies are promising agents for use in cancer treatment. However, passive immunotherapy could gain in efficacy in several cancer types by increasing the number of responding patients or decreasing disease recurrence. Many transcriptomic datasets for multiple subtypes of cancers have recently become publicly available, making possible detailed genome-wide analysis and comparisons with normal references.

**Objectives:** We describe here a new method for identifying potential cancer antigens that would be accessible to antibodies in the extracellular milieu and encoded by genes specifically overexpressed in cancers.

**Design:** This identification can be broken down into four key phases: (a) The annotation of transmembrane proteins; (b) the analysis of gene overexpression in specific cancers; (c) the prioritization of the most relevant proteins and (d) biological validation. We focused on the first three steps with the development of a method applicable to all types of cancers.

**Results:** We first tested various methods for annotating transmembrane proteins. We applied the best of these methods to the Uniprot dataset, and developed a fast, simple algorithm for analyzing the overexpression of genes, taking tumor heterogeneity into account. We then validated the complete method on a large publicly available breast cancer dataset. Seven of the 13 potential cancer antigens found are known to be involved in breast cancer, and one is the target of new drugs currently in development. Our method is available as an R package.

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**SESSION VI—TRANSLATING PHARMACOGENOMICS INTO CLINICAL MEDICINE**

**Digital signatures of drug response as clinical decision tools**

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The development of omics-based approaches to predict an individual’s drug response is one of the visions of personalized medicine (1) New generation sequencing and microarrays enable accurate and inexpensive measurement of all presently known genetic components of interindividual variability in drug response. However, clinical implementation of pharmacogenomic testing has not been widely adopted to improve patient care. One reason is that in order to guide prescribing decisions, preemptive pharmacogenomic test results should be available to physicians.

For an increasing number of actionable gene-drug interactions, practice guidelines based on genetic and clinical information have been established (2). The incorporation of pharmacogenetic test results combined with clinical decision support (CDS) into machine-readable electronic medical records (EMRs) allows clinical implementation of this information. Results of preemptive testing in several institutions with up to 12 pharmacogenes and approximately 30 “high risk” drugs indicates that a) More than 90% of patients carry at least one “high risk” diplotype. b) The digital signature of the drug response profile of the patient is available at the point of care. c) Preemptive pharmacogenomic testing improves drug prescribing in the clinic. In the future, the question may not be whether to order a pharmacogenomic test but how to best use the already existing genomic test results.

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**References**


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**A proposed model for translating pharmacogenetics into the clinical setting**

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**Background:** The number and power of pharmacogenetic studies have grown significantly in the last decade, creating a comprehensive evidence base linking genotypes to variations in drug response. The debate has recently moved from whether the evidence surrounding pharmacogenetics is valid to how we can interpret this information for clinical use. Little is known about prescribers’ opinions towards pharmacogenetics in the UK even though they will eventually be the key community using pharmacogenetic data to improve patient outcomes.

**Objective:** To present a model suggesting best practice guidelines for the translation of pharmacogenetic data into a clinical setting. This model encompasses education of healthcare professionals, digitalization of patient data, improved understanding for prescribers and better uptake of pharmacogenetic advice in clinical settings.

**Design:** The model is based on the outcomes of a quantitative study that will be conducted on a small to mid-sized group of prescribers. This study will compare paper prescribing to a digital solution with integrated pharmacogenetic prescription advice. Key metrics will include time taken to prescribe, ease of use, prescriber engagement and number of adverse drug events predicted by post-prescription analysis.

In addition, participants will complete a qualitative questionnaire on the presentation of pharmacogenetic information.

**Results:** Preliminary investigations reveal a need for further education amongst prescribers with regard to pharmacogenetic warnings relating to adverse events, and pharmacogenetics in a broader sense. Whilst geneticists are advancing their understanding of genomics and disease, this information is not easily translated to clinical settings and there are currently not established guidelines. We reserve further detail of the trial outcomes until a complete analysis has been conducted.

**Conclusion:** The gap between research and clinical use remains a significant challenge for the adoption of pharmacogenetics as a standard part of medical practice. By presenting results from a study of individual prescribers, and an extrapolated set of high-level guidelines, we aim to provide a useful framework that is accessible to healthcare professionals at all stages. Findings from the study should help drive better clinical outcomes for prescribers at the point of care.

Gene polymorphisms associated to clinical and biological response to Infliximab in Crohn Disease.

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**Background:** Infliximab (IFX), a chimeric mouse/human monoclonal immunoglobulin-G1 antibody against tumor necrosis factor-alpha (TNF-α), whose efficacy in Crohn Disease (CD) can be evaluated by clinical biological markers. Several single nucleotide polymorphisms (SNPs) have been associated to IFX efficacy for CD in several studies.

**Objectives:** To evaluate the clinical efficacy of IFX for CD and the association of SNPs on different genes in recently published clinical trials.

**Design:** Literature review of studies published in the period 2006-2013 focused on association of SNPs and IFX efficacy. The assessment of effectiveness was determined considering the following endpoints: Crohn Disease Activity Index (CDAI) and C-reactive protein (CRP) levels.

**Results:** Five clinical trials were included, evaluating the following genes: TNFRSF1B (TNF-alpha receptor IB), TNFRSF1A (TNF-alpha receptor 1A), FCGR3A (FcyRIIIB-NA1, FcyRIIIB-NA2 isofoms of FcyRIIIB receptor and FcyRIIA receptor (CD16)) and IL1B.

Two SNPs in TNFRSF1B were found to be associated with good response to IFX: G-allele for rs976881 and G-allele for rs1061622.

In Japanese patients, A-allele for rs767455 (TNFRSF1A) showed efficacy to IFX, with 73% response vs 50%.

V/V-genotype for FCGR3A (rs396991) showed better biological response in Caucasians and Japanese patients. This SNP also showed higher antibody-dependent cell-mediated cytotoxicity (ADCC) against TNF-alpha expressing cells, due to a higher binding affinity for IFX in V/V cells.

FcyRIIIB-NA1 achieved 65% response vs 35% for FcyRIIIB-NA2.

C-allele for IL1B (rs1143634) was associated with higher serum IL1B concentration and non-response (86.4% vs 65%).

**Conclusion:** G-allele for TNFRSF1B (rs1061622), G-allele for TNFRSF1B (rs976881), A-allele for TNFRSF1A (rs767455), V/V-genotype for FCGR3A (rs396991) and FcyRIIIB-NA1 are associated efficacy to IFX.

V/V-genotype for FCGR3A (rs396991) may be utilized as a possible predictor for biological response to IFX treatment.

C-allele for IL1B (rs1143634) may be a possible predictor of inefficacy, representing an inexpensive feasible alternative to conventional cytokines determination.


Pharmacogenomics of oral antidiabetic medications

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**Background:** Type 2 diabetes mellitus (T2DM) is an increasingly prevalent disease that has reached epidemic proportions. Several classes of drugs are currently available to treat T2DM patients, however, clinical response to these drugs often exhibits significant variation among individuals. Interindividual variability in response to antidiabetic drugs is affected by genetic polymorphisms.

**Objective:** To present the current state-of-the-art in the pharmacogenomics of antidiabetic oral medications. Specifically, the association of specific gene polymorphisms with the interindividual variability in the therapeutic and adverse reaction effect of the oral antidiabetic medications metformin and sulfonylureas.

**Results/Conclusions:** Gene polymorphisms in OCT1, OCT2, MATE1 and near ATM gene region are strongly associated with response to metformin, while CYP2C9 and POR gene polymorphisms are associated with disposition, response and adverse events incidence during therapy with sulfonylureas. Several ongoing clinical trials registered in ClinicalTrials.gov website are assessing the effect of gene polymorphisms with antidiabetic drug response. It is anticipated that the results of these trials will strengthen currently available evidence on the usefulness of oral antidiabetic pharmacogenomics and help clinicians in effective prescribing of oral antidiabetic medications.

TOMM40 poly T variants modify the pharmacogenetic response to conventional treatments in Alzheimer’s disease


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The TOMM40 locus is located adjacent to and in linkage disequilibrium with APOE on 19q13.2. A polyT repeat in an intronic polymorphism (rs10524523)(intron 6) in the TOMM40 gene, which encodes an outer mitochondrial membrane translocase involved in the transport of amyloid-β and other proteins into mitochondria, has been implicated in Alzheimer’s disease (AD), and APOE-TOMM40 genotypes have been shown to modify disease risk and age at onset of symptoms.

We have performed the first genetic screening of the TOMM40 gene in the Spanish population and studied the influence of poly T variants on the therapeutic response to a multifactorial treatment for one year in 920 patients with AD. The frequency of the TOMM40-poly T1 repeats (allele 1)(short variant) was T1-45 0.87%, T1-64 64.35%, T1-22 5.87%, T1-29 2.72%, T1-30 10.54%, T1-31 1.74%, T1-34 1.30%, T1-35 6.52%, T1-36 5.33%, and T1-37 37%; the frequency of the TOMM40-Poly T2 repeats (allele 2)(large variant) was T2-45 0.43%, T2-16 18.15%, T2-22 4.02%, T2-29 5.33%, T2-30 10.43%, T2-31 1.20%, T2-33 0.87%, T2-34 11.30%, T2-35 13.59%, T2-36 25.0%, T2-37 6.20%, T2-38 1.20%, T2-39 0.76%, and T2-40 1.52%. The responders rate (MMSE score at 12 months higher or equal to baseline levels) was 59% (females, 60%; males, 59%). Globally, AD patients improved their cognitive performance for the first 9 months of treatment (p<0.05), showing a progressive mental deterioration thereafter. The best responders were carriers of the TOMM40 T1-35 (73%), T1-37 (71%), T1-36 (63%), and T1-16 variants (62%), and the worst responders were those
patients harbouring the T1-15 (13%) and T1-29 variants (32%). A high concentration of APOE-3/3 carriers was found among responders; in contrast, patients with the APOE-4/4 genotype concentrate among TOMM40 poly Ti-related non-responders, indicating that the association of specific APOE and TOMM40 variants is responsible for the pharmacogenetic outcome in patients with AD.

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Individual Approach for Predictive Genetic Testing by Help Decision Making System: Application for Pharmacogenetics

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The International Human Genome Project was completed in 2003, what has symbolically announced the beginning of post-genomic era in the third millennium. Due to completion and rapid development in the field of genetics, and molecular medicine which has accelerated the use of predictive and personalized medicine/healthcare in routine medical and pharmacy practice for more efficient treatment of individuals. Genomics is one of the key technologies enabling personalized medicine/healthcare and the broader field of theranostics. Pharmacogenomics which studies genetic variations among individuals to predict disease susceptibility and responses to therapeutic agents arose in response to such recognition and can be regarded as the 21st century’s answer for the rational use of drugs—the right drug to the right patient. Lack of knowledge among clinicians regarding pharmacogenetics is often cited as one of the barriers delaying its clinical uptake, albeit there are many other, more crucial aspects that impede the implementation of pharmacogenetics into routine medical practice. The most important barriers delaying clinical uptake and application of pharmacogenomics is lack of knowledge and insufficient education of health professionals regarding pharmacogenetics and genomics rather than technical issue. This aspect should be explored more in developing countries. Moreover, there is a lack of qualification of information concerning to pharmacogenetic testing results; the pharmacogenetic testing results without precise personalized interpretation regarding to patient’s peculiarities, such as his/her lifestyle factor, like smoking, or other nutritional status and his/her clinical data could not be useful for medical professionals and patients. To this end Expert system (ES) is a highly personalized help decision making system for interpretation of pharmacogenetic and other predictive genetic testing. ES allows to perform more precise analysis and diagnosis for each individual patient according to his peculiarities: life style factors, anamnestic and clinical data. In conclusion, ES provides physicians and pharmacists with explanation, clinical biological interpretations and advice, which helps to obtain a global vision of the patient’s status. It also opens new opportunities for statistical analyses and mathematic modeling for investigation of possible new trends in different populations due to environment-gen interactions.

Pharmacogenetic characterization of the Kosovar Albanian Population

C Nofziger*, L Raka†, A Baruti*, G Scantamburlo* and M Paulmichl†
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Background and Objectives: Genetic profiling targeted to pharmacogenetics individualizes the management of treatment strategies in the context of a unique patient predisposition to disease. Through this paradigm, health care providers will be able to offer highly effective, precision-targeted therapies and reduce adverse drug reactions (ADRs). Little information is available on the distribution of single nucleotide polymorphisms (SNPs) known to influence the functionality of genes affecting pharmacokinetic (PK) parameters in the Kosovar Albanian Population. In this population, the prevalence of diseases requiring the use of drugs for which genetic profiling is important, is staggering, i.e. one study found prevalence rates as high as 25% for post-traumatic stress disorders (PTSD), a value considerably higher than the 18% incidence found in U.S. soldiers returning from combat in the Iraq war.

Design: Whole blood samples from 100 Kosovar Albanians (for at least two generations resident from the same area) were taken (written consent and study approved by the local ethic commission #05-9/3) and genomic DNA (gDNA) isolated using standard protocols. The gDNA was used as a template for qPCR employing the TaqMan OpenArray pharmacogenomics (PGx) panel on the QuantStudio 12K Flex platform and analyzed using TaqMan Genotyper software (both, Life Technologies, Carlsbad, California, USA).

Results: Results will be reported for 12 phase I, 6 phase II and 11 transporters. For these 29 PK related genes, 158 individual SNPs were measured. In addition, the copy numbers for CYP2D6 were also determined.

Conclusions: A highly flexible, accurate and robust array-platform was used for determining PGx relevant SNPs in a precisely defined population of European origin.


Presentation of the ESPT Pharmacogenomics Network strategy

Ron Van Schaik, Rotterdam, The Netherlands
**LIFE TECHNOLOGIES LUNCH SYMPOSIUM**

**GENOMIC TOOLS**

*Chair:* Peter Jacobs, Life Technologies, Belgium  
*Speaker:* Charity Nofziger

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**26 September 2014 – Afternoon**

**SESSION VII–PROTEOMIC BIOMARKERS IN SYSTEMS PHARMACOLOGY AND MEDICINE**

**Systems glycobiology for understanding the underlying mechanism of disease onset, biomarker and therapeutics**

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**Background:** Our group has been focused on the biological role of glycosyltransferases involving the biosynthesis of N-glycan branching and identification of their target proteins in relation to various diseases such as emphysema, diabetes and cancer metastasis (1) and also established the simultaneous method of analysis of nucleotide sugars, donor substrates for glycosyltransferases.

**Objective:** In order to understand glycan structure and function from the viewpoint systems medicine, we aim the integrative and dynamic analysis of various components of a functional unit of glycans designated as “glycan cycle”.

**Design:** In order to investigate the fate of UDP-GlcNAc, a donor substrates of various GlcNAc transferase, we developed a tracing method for UDP-GlcNAc synthesis and utilization, and GlcNAc utilization using $^{13}$C$_2$-glucose and $^{13}$C$_3$-glucosamine, respectively, followed by the analysis of mass isotopomers using liquid chromatography-mass spectrometry.

**Results:** Metabolic labeling of cultured cells with $^{13}$C$_2$-glucose and the analysis of isotopomers of UDP-HexNAc (UDP-GlcNAc plus UDP-GalNAc) and CMP-NeuAc revealed the relative contributions of metabolic pathways leading to UDP-GlcNAc synthesis and utilization. In pancreatic insulinoma cells, the labeling efficiency of a $^{13}$C$_2$-glucose motif in CMP-NeuAc was lower compared with that in hepatoma cells.

Using $^{13}$C$_2$-glucosamine, the diversity of the labeling efficiency was observed in each sugar residue of N- and O-glycans on the basis of isotopomer analysis. In the insulinoma cells, the low labeling efficiencies were found for sialic acids as well as tri- and tetra-sialo N-glycans, whereas asialo N-glycans were found to be abundant. Essentially no significant difference in secreted hyaluronic acids was found among hepatoma and insulinoma cell lines. This indicates that metabolic flows are responsible for the low sialylation in the insulinoma cells. Our strategy would be useful for systematically tracing each stage of cellular GlcNAc metabolism.

**Conclusion:** Thus systems glycobiology is one of the promising approaches for understanding the role of glycans in disease onset, biomarker and therapy.

**Acknowledgements:** This study was supported by the grant-in-aids for scientific research (A) from the JSPS.


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**Personalized medicine in cardiovascular disease**

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With the beginning of the new century – in 2000 when it was announced that the human genome had been sequenced, a big hype about the upcoming impact of personalized medicine in cardiovascular disease started. But it took until 2003 to roughly complete 92% of the full human genome sequence, with a few unsequenced gaps which are difficult to resolve remaining. By now all protein-coding sequences seem to be known. However, the initial enthusiasm that SNP or haplotype-based testing might pave the way for an individualized tailored medicine cooled down, once genotype-guided controlled studies did not translate into a significant benefit in daily routine care.

By today, it has been realized that the universal dogma of DNA, gene, transcription, and protein has changed from one gene, one protein, one phenotype to many genes, many proteins, and many phenotypes. New “players” are on the field today with epigenomics for scientific research (A) from the JSPS.

Thus, from this perspective, it is not surprising that genotype-guided therapies do not belong to today’s mainstream cardiovascular care. This, does however, not necessarily mean such an approach has failed. It took almost 100 years in cardiovascular care to acknowledge the causal impact of elevated LDL-cholesterol in the development of atherosclerosis with subsequent detrimental clinical outcomes such as ischemic stroke or myocardial infarction. It is only 14 years ago, that the human gene was announced to be sequenced. From that perspective, possibly it will take another decade or longer until we see a genetics/genomics-based approach for personalized medicine in “mainstream” cardiovascular care.

Pharmacogenetics and –genomics is mainstream today in drug development and translational medicine, even large phase III trials in cardiovascular medicine investigate large subsets of patients for genetic and genomic markers to predict individual therapeutic response or vice versa adverse reactions to drug treatment. Furthermore, the cardiovascular field is somehow trailing behind oncology with respect to the impact of genetic and genomic testing in routine patient care. Thus, today’s population-based approach in large clinical trials, where efficacy and safety is dealt with as the statistical difference between the two Gaussian curves of the old versus the new therapy, will evolve into a personalized approach sooner or later once the respective markers have been identified.
Proteomic Approaches For Biomarker Identification In Chronic Lung Allograft Dysfunction (CLAD)

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Background: With 39,000 procedures performed in the last 30 years, lung transplantation (LT) has become a validated treatment for terminal lung diseases. The long term survival after LT remains limited because of Chronic Lung Allograft Dysfunction (CLAD) onset, affecting 50% of LT recipients within 5 years post-LT. This condition associates Bronchiolitis Obliterans Syndrome (BOS) and Restrictive Allograft Syndrome (RAS) and its diagnosis, based on the non-specific decline of respiratory function, is only possible after the onset of irreversible damages.

Objectives: As a part of an EU-funded FP7 project named SysCLAD, this study aimed to identify biomarkers at 6 and 12 months after LT, that will predict CLAD onset 3 years later, by 2 complementary proteomic approaches.

Design: BALF (bronchoalveolar lavage fluid) and plasma from the 100 first patients of COLT cohort who reached 3 years post-transplantation were selected for analysis. iTRAQ-MALDI-TOF/TOF MS and MS/MS approach was first performed for identification and quantification of biomarkers after pooling samples according to patients’ phenotype (BOS, RAS, Stable). Then, SELDI-TOF MS approach completed proteome description with information on individual proteomic profiles.

Results: BALF composition in stable LTR reflects the infiltrate of allo-immune and non allo-immune pro-inflammatory mediators balanced by antiprotease defenses. In patients who will develop a CLAD, an imbalance in favor of pro-inflammatory mediators and a loss in tissue repair processes were observed during the first year post-LT, before irreversible damages onset.

Conclusion: All results will be integrated into a predictive computational model of CLAD based on clinical and experimental data: environment, phenotype, microbiome, immunological assays and omics.

From synovial tissue biopsies to blood and bone marrow transcriptomes in biomarker discovery of rheumatoid arthritis

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Background: Chronic and fluctuating levels of inflammation are main characteristics of patients with rheumatoid arthritis (RA). A main challenge in disease-management of RA is to establish objective criteria relevant for diagnosis and therapeutic stratification of patients.

Objective: To decipher inflammation and determine heterogeneity between RA patients, we generated and analysed cell- and tissue-specific transcriptomes from different body compartments and selected candidate markers for validation at the protein level.

Design: Affymetrix HG-U133Plus-arrays were used for generation of gene-expression profiles from synovial tissue biopsies and monocytes from blood and bone marrow of patients with RA and osteoarthritis (OA)(1). Multiplex-immunoassays and ELISA were used for validation of 28 candidate markers at the protein level in synovial fluid (SF) and matched serum samples from RA and OA patients.

Results: Comparisons between RA and OA monocytes from bone marrow and blood revealed only a minor overlap, indicating that RA-profiles of these two body compartments are tissue-specific. The RA-profile generated by comparison of synovial tissues (ST) from RA and OA patients identified increased expression of genes, which indicates infiltration but also activation of various immune cells. The RA-profile from ST disclosed many monocyte-related genes, and interestingly these genes were not related to the monocyte activation in blood and/or bone marrow in RA patients. RA-profiles from all three body compartments were used for selection of candidate markers. Protein measurement in SF largely resembled transcriptome data. When measured in serum, only 10 out of 28 markers reached statistical significance. Interestingly, their combination was able to identify differences between RA and OA, but also heterogeneity between RA patients.

Conclusions: Cell- and tissue-specific transcriptomes of different body compartments are an exceptional source for selection of biomarkers, which can be detected in serum. Although far weaker than in SF, these markers suggest objective scoring for disease-management in RA.

Acknowledgements: The work was supported by the ArthroMark, by the EU FP6 project AutoCure, and by the IMI JU funded project BeTheCure.


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SESSION VIII—METABOLOMIC TOOLS FOR CLINICAL IMPLEMENTATION OF PERSONALIZED MEDICINE

Recent development in metabolomics for diagnostics and drug discovery

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The drug discovery and development process is a more and more risky activity. It is well documented that the innovative drugs in development are less and less abundant for an higher cost. There is a tremendous need to use new methods for increasing the rate of success of the research pipe line. Metabolomics beside the others omics (genomics, proteomics...) is very promising in order to address major life science trends and challenges.

Metabolomics is able
- to provide high-throughput, quantitative technologies for biochemical phenotyping of large sets of samples (human epidemiological cohorts),
- to identify metabolites in human biofluids and cell extracts,
- to develop large-scale flux profiling and sub-cellular fluxomics through integration of analytical data from multiple analytical sources,

One of the main interests in drug development is to provide relevant biomarkers for personalized medicine and diagnosis of diseases with major socio-economic impact such as obesity, cancer, cardiovascular, neurological pathologies and infectious diseases.

Recent examples from the literature will be presented and discussed.

Metabolomics for personalized medicine and patient stratification

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The metabolome is the set of small molecular mass compounds found in biological media, and metabolomics, which refers to as the analysis of metabolome in a given biological condition, deals with the large scale detection and quantification of metabolites in biological media. It is a data driven and multidisciplinary approach combining analytical chemistry for data acquisition, and biostatistics, informatics and biochemistry for mining and interpretation of these data. Since the middle of the 2000’s, high resolution mass spectrometry (HRMS) is widely used in metabolomics, mainly because the detection and identification of metabolites are improved compared to low resolution instruments. Furthermore, thanks to their versatility, HRMS instruments are the most appropriate to achieve an optimal metabolome coverage, at the border of other omics fields such as lipidomics.

The aim of this lecture will be to present HRMS based tools for metabolomics and lipidomics, and their relevance to the field of biomarker discovery for the diagnosis and follow-up of pathologies. This will be done through studies developed at the laboratory, mainly in the field of rare and neurological diseases.

A Metabolomic Approach for Biomarker Discovery and Diagnostic Development in Diseases Related to Obesity and Cancer

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Metabolomics is defined as “the non-biased quantification and identification of all metabolites present in a biological system” but in practice the term metabolomics is used in a rather broad sense and covers many different analytical methodologies. To address the challenges associated with metabolomics, a comprehensive, integrated analytical and data handling platform was developed that provides a chemo-centric global metabolomics analyses of biological systems. The analytical platform incorporates three UHPLC/MS/MS2 accurate mass methods and a GC/MS method which increases the overall coverage of small molecules in a biological sample. This integrated platform allows the robust, high-throughput collection and analysis of analytical data and accurately identifies a large number and broad spectrum of biochemicals thereby facilitating biochemical interpretation of metabolomic experiments.

Using our metabolomics approach we have focused on two major disease areas, diseases associated with obesity and urological cancer. In very large cohorts of patients, we have discovered biomarkers for both insulin resistance and impaired glucose tolerance. We have developed diagnostic tests based on these results and have commercialized both tests. We are now developing tests for liver disease, and kidney disease which are also closely linked to obesity. The general idea is to develop tests for the early stage of the obesity related diseases for early detection and early intervention.

In over 400 studies in cancer, we have been able to establish the principal biochemical changes associated with the derangement of metabolism that leads to metastasis. Using this approach and our understanding of cancer related biochemical pathways, we have determined the mode of action of anti-tumor compounds as well as identifying biomarkers related to the onset of prostate cancer. These biomarkers have now been developed into a diagnostic test for better detection of cancer positive patients in digital rectal examination-negative patients with intermediate levels of PSA.

Where is Wally? – Untargeted metabolomics in inborn errors of metabolism

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The body fluid metabolome at any point in time is influenced by the endogenous metabolism, by the gut microbiome, by food intake and also by the medication given by the clinician. The analytical challenge is to find the relevant biomarker(s) that reflect the fingerprint of a specific disease and provide information about the course of the disease. We have shown that our approach with LC-Qtof MS detects a very broad range of small molecules in the complex matrix of the body fluid. Sensitivity limits are in the nanomolar range. Our work shows that even in an individual person the relevant biomarker profile can be picked up without prior knowledge of underlying disease or condition. The basis for this is a robust liquid chromatography system with the Qtof as a sensitive detector.
Levels of xanthurenic acid, a putative activator of type 2/3 metabotropic glutamate receptors, are reduced in the blood of schizophrenic patients and their healthy relatives

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The kynurenine pathway of tryptophan metabolism has been implicated in the pathophysiology of neurological and psychiatric disorders, including schizophrenia. Metabolites of this pathway, such as kynurenic acid, quinolinic acid, cinnabarinic acid, and xanthurenic acid are known to interact with ionotropic and metabotropic glutamate receptors, thereby influencing excitatory neurotransmission in the CNS. Levels of kynurenic acid were found to be increased in the CSF and brain tissue of schizophrenic patients as a result to a reduced activity of kynureninase (KMO), the enzyme which converts kynurenine into 3-hydroxykynurenine. This evidence is in line with the hypothesis of a glutamatergic hypofunction in schizophrenia because kynurenic acid is an antagonist at the glycine site of NMDA receptors. Whether levels of other metabolites of the kynurenine pathway are altered in schizophrenia is unknown at present. We developed an HPLC/MS-MS assay that allows a reliable estimation of all metabolites of the kynurenine pathway. Using this method, we found a strong reduction in blood levels of 3-hydroxykynurenine and xanthurenic acid, and a significant increase in blood levels of anthranilic acid in a large cohort of schizophrenic patients, as compared to age-matched healthy controls. First-degree relatives of schizophrenic patients had levels of xanthurenic acid intermediate between those found in schizophrenic patients and healthy subjects. Unexpectedly, blood levels of kynurenic acid were unchanged in schizophrenic patients. Xanthurenic acid is a putative agonist of group-II metabotropic glutamate receptors (mGlu2/mGlu3 receptors), the activation of which relieves psychotic symptoms in experimental animals and humans. Thus, a reduced formation of xanthurenic acid might contribute to the pathophysiology of schizophrenia, although the precise source of blood xanthurenic acid (liver or other peripheral organs vs. CNS) remains to be determined.


Memory genes and post-traumatic stress disorder

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Strong memory of a traumatic event is thought to contribute to the development and symptoms of posttraumatic stress disorder (PTSD). Therefore, a genetic predisposition to build strong memories could lead to increased risk for PTSD after a traumatic event. We have shown that variability in several genes is associated with memory capacity—including aversive memory—in nontraumatized subjects of European descent. Genetic variability is also associated with brain activity during encoding of emotionally aversive information. Finally, the identified genetic variants are also related to traumatic memory and to the risk for PTSD in heavily traumatized survivors of the Rwandan genocide. Our results suggest a genetic link between memory and the risk for PTSD.
27 September 2014 – Morning

SESSION IX–HUMAN NUTRITION, ENVIRONMENT AND HEALTH

Systems Approaches to Define and Maintain Health

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At the Nestlé Institute of Health Sciences (NIHS), we take a systems biology approach to better understand and act upon healthy ageing and disease prevention with a focus on metabolic, cognitive and intestinal health. Disease phenotypes of central interest to us are Alzheimer’s, diabetes and gastrointestinal inflammation.

Natural human cell models (iPSCs) help guide us to key metabolic phenotypes and nutritional interventions tested in human clinical studies, which are not only performed in the classical case/control- but also in the longitudinal design incorporating challenges to hemostasis. Mainly, but not exclusively deep sequencing-based functional genomics and nuclear magnetic resonance plus mass spectrometry-based proteomics/lipidomics metabonomics/micronutrient analysis are giving us the necessary holistic mechanistic insights into the host and gut microbial metabolism [1]. Ultimately we translate this basic science into personalized solutions at the interface between food, pharma and diagnostics.

Our omics platforms generate a systems view on the metabolic trajectory of natural human cell lines, animal models and human subjects. The emphasis lies on “trajectory” as we monitor these cells, animals and human subjects not only in groups at a given moment in time but also longitudinally, i.e. over time, with every biological entity functioning as its own case/control pair, rather than comparing a case to a control group by taking omics snapshots at a given moment in time [2].

A further, innovative angle of our research into metabolic, cognitive and intestinal health is to assess metabolic elasticity and flexibility rather than comparing systems at phases of homeostasis: challenging biological systems repeatedly over time and monitoring their (failure of) oscillation back to normality are giving us early insights into possible deviations from healthy metabolic trajectory and open windows of preventive opportunity [2].

References

[1] Kussmann M, van Bladeren PJ; Frontiers Genetics 2011 (2) 21, 1-12: “The extended nutrigenomics-understanding the interplay between the genomes of food, gut microbes and human host”.

Nutrigenomics of Metabolic Health

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The progress in and success of biomedical research over the past century was built on the foundation outlined in R.A. Fisher’s The Design of Experiments (1935), which described the theory and methodological approach to designing research studies. A key tenet of Fisher’s treatise, widely adopted by the research community, is randomization, the process of assigning individuals to random groups or treatments. Comparing outcomes or responses between these groups yields “risk factors” called population attributable risks (PAR), which are statistical estimates of the percentage reduction in disease if the risk were avoided or in the case of genetic associations, if the gene variant were not present in the population.

High throughput metabolomics, proteomic and genomic technologies provide 21st century data that humans cannot be randomized into groups: individuals are genetically and biochemically distinct. Gene – environment interactions caused by unique dietary and lifestyle factors contribute to heterogeneity in physiologies observed in human studies. The risk factors determined for populations (i.e., PAR) cannot be applied to the individual. Developing individual risk or benefit factors in light of the genetic diversity of human populations, the complexity of foods, culture and lifestyle, and the variety of metabolic processes that lead to health or disease are significant challenges for personalizing dietary advice for healthy or medical treatments for individuals with chronic disease. We describe the results of the Delta Vitamin pilot study and the conceptual basis of the Brazil Micronutrient Studz that analyze individual responses to interventions. The strengths, weaknesses, and implications of the results will be discussed.

For Heart disease genetics / expression & epigenetics associations with fat related phenotypes

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Non-alcoholic fatty liver disease. Genetic etiology and nutritional intervention

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Background: Non-alcoholic fatty liver disease (NAFLD) is defined as the hepatic manifestation of the metabolic syndrome and thus it is mainly linked to excess body weight and systematic insulin resistance. Moreover, there is an abundance of published data indicative of the contribution of environmental factors and susceptible genetic background in NAFLD onset and progression. PNPLA3 rs738409 is the main variant studied for NAFLD and diet is recognized as the drastic environmental exposure. Design: Greek adults (n=400) were recruited and monitored for NAFLD status [absence (26.4%), low (40.8%), medium (27.2%), high (7.6%)] using abdominal
ultrasonography. They were interviewed for demographic data, medical history and dietary intake was assessed by a 172-food item questionnaire. Anthropometric and biochemical measurements were collected. Results: The progression of the disease was associated with higher body mass index, % fat mass, waist circumference, waist-to-hip ratio, triglycerides, glucose, ALT and AST/ALT levels (p < 0.05). Dietary intakes of starch food, meat, fish, fruits, vegetables, soft drinks and dairy products were assessed. Moderate fruit (2.6 portions/day) fish (1.7 portions/week) and diary products (1.15 portions/day) consumption was negatively correlated with the disease progression when adjusted for age, gender and calorie intake (p < 0.05). However, higher cheese intake (2.7 portions/day) were associated with worse disease status (p < 0.05). Patients of medium and high lipid infiltration were selected to follow a 6-month intervention trial with Corinthian Raisin (CR). In parallel a control group followed commonly given recommendations for therapeutic lifestyle changes. This 6-month intervention study included 60 males and females with NAFLD, matched for age and body mass index (BMI). Subjects are randomized into two groups to receive 36g of CR daily (n=30) or control (n=30), along with counseling for therapeutic lifestyle changes. Ultrasound scanning and shear-wave elastography will be applied as diagnostic tools at baseline and to assess the effect on liver fibrosis at the end of the trial. Medical history, anthropometric indices, physical activity data, biomarkers, plasma fatty acid profile will be collected for all the participants at baseline, 3 months and at the end of the trial. Conclusions: The liver ultrasound revealed a high incidence of NAFLD in Greek volunteers. The disease status was predicted by classical anthropometric and biochemical risk factors. Different food groups’ intake protected or worsened the disease status and finally the results of the diet intervention trial with the genetic data will be assessed to monitor the disease.


Epigenetics in Rheumatic Diseases

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Epigenetics is regulating the expression of the genetically encoded information. This highly complex regulatory network includes acetylation, methylation, phosphorylation, sumoylation and non-coding RNAs (ncRNA), such as PIWI, snoRNA, miRNA and IncRNAs (1). Since these epigenetic processes are highly intercalated, our laboratory is exploring in complementary working research teams these individual regulatory processes in rheumatic diseases (2) as part of the public-private initiative IMI (Innovative Medicine Initiative) within the 35 Mio Euro funded research project BTCure (http://btcure.eu/). Of especial interest are the environmental risk factors in autoimmune diseases, such as smoking which is leading to long persisting epigenetic modifications, such as the induction of specific sirtuins (3). In numerous rheumatic diseases, like rheumatoid arthritis (RA), SLE, OA, SSC and pulmonary hypertension novel diagnostic signatures are emerging, i.e. miR signatures in RA (4). Also novel therapeutic strategies are emerging by modulating the methylation in RA (5) or by the inhibiting of specific bromodomains.

Reference:


Is obesity a decanalized phenotype? Analysis of genetic variation in obesity genes in worldwide populations

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Adaptation to new environment during early human migrations played the substantial role in shaping the genetic structure of modern human populations. Since the thrifty genes hypothesis by James Neel (1962), obesity-related phenotypes have been considered as traits depended on gene-environment interaction. Recent hypotheses of ancestral susceptibility (di Renzo, Hudson, 2005) and decanalization (Gibson, 2009) may provide further conceptual bases on the evolutionary origin and population prevalence of obesity and related traits. We have investigated the distribution of SNPs associated with obesity according to recent genome-wide association studies (GWAS) in worldwide populations. Twenty GWAS SNPs were genotyped in 11 populations from Europe, Central Asia and North-East Asia. Data on allele frequencies in 5 unmixed HapMap populations from Africa and South Asia were also included into analysis. The analyzed genetic markers are divided into two equal groups characterized by principally different patterns of genetic diversity. First group of markers (10 SNPs) demonstrate significant correlations of allele frequencies with key climatic parameters of the populations; accumulation of positive signals of natural selection; and systematic decrease of ancestral allele frequency from Africa to Eurasia. This part of genetic component of obesity may be considered as non-neutral and decanalized by natural selection during human dispersal out of Africa. Second part of obesity genetic markers follows the features of neutral genetic variability, i.e., is not correlated with climate, does not demonstrate signals of natural selection and systematic pattern of allele frequencies. “Decanalized” and “neutral” sets of SNPs are also different in their total genetic diversity and differentiation patterns:
“decanalized” SNPs as opposed to “neutral” ones have significantly higher average Fst levels and show the decrease of genetic diversity, measured as expected heterozygosity, from Africa to Eurasia, unlike “neutral” SNPs and opposite to trend for genome-wide genetic variation. Thus we may conclude that observed characteristics of worldwide frequency spectrum in obesity-associated genes may be, at least partially, explained by the hypothesis of canalization/decanalization of genotype-environment relationships under the pressure of natural selection.

Abdominal obesity responsible for depression in adolescents and youth

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Background: Central adiposity is the source of inflammatory factors, causing endothelial dysfunction (ED). ED in kidneys results in microalbuminuria. In central nervous system, inflammatory factors mediate neurotransmitter metabolism promoting excitotoxicity and oxidative stress, and a major clinical symptom in that state is depression.

Objective: To examine the relationship of depression with visceral obesity and other metabolic syndrome (MS) criteria, blood pressure, lipids, inflammation and endothelial dysfunction factors.

Design: Cross-sectional study.

Methods: The study included 49 obese male individuals with pre-MS or MS (age 16-30) classified into two groups: I—with depression; II—without depression. Depression was rated by Hamilton scale as moderate, severe and very severe. ATP III classification was applied for diagnosing MS. Patients with less than three above criteria were considered pre-MS. The following parameters were observed: BMI, waist circumference (WC), blood pressure, lipids, CRP, microalbuminuria. OGTT (0, 30 and 120 minute glycaemia and insulin) was used to evaluate the extent of disorder and determine the insulin mean value.

Results: Depression was found moderate in 32.5% and severe in 7.5% individuals. Results were as follows: body weight: I-106.1±23.8, II-98.6±20.5 kg; BMI: I-34.4±7.4, II-32.7±6.5kg/m2; WC: I-106.9±18.5, II-102.8±15.4 cm; blood pressure: I-128.8±9.3 / 85±8.1, II-120.4±14.6 / 79±11/mmHg; HDL: I-1.18±0.2, II-1.25±0.3mmol/l; triglycerides: I-1.6±1.17, II-1.8±1.07 mmol/l; mean value of insulin: I-7.8±6.0, II-6.3±2.26 μU/l; CRP: I-5.6±6.3, II-3.8±4.4 mg/l, microalbuminuria: I-34.2±35.5, II-11.3±6.1mg/24h. Correlations: depression with systolic pressure (p<0.05). A statistically important difference between groups was found for microalbuminuria and systolic pressure (p<0.05).

Conclusion: Abdominal obesity accompanied with hyperinsulinemia, insulin resistance, lipid status alteration, hypertension, inflammation factors and microalbuminuria was more pronounced in youth with depression; this was also confirmed by depression being in correlation with systolic pressure and microalbuminuria. Chemokines and cytokines produced by white adipose tissue may contribute to widespread immune activation, potentially causing or exacerbating diseases associated with inflammation such as type 2 diabetes, cardiovascular disease, cancer and depression. Depression correction occurring after abdominal obesity, insulin resistance and other before mentioned metabolic syndrome parameters being reduced by Mediterranean diet confirms that visceral obesity and depression are highly connected.


ENVIRONMENT AND HUMAN HEALTH

The human exposome and contaminant mixture effects

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Humans are exposed to a variety of insults that may have significant health effects. These include a variety of chemical, physical, biological as well as social and psychological stressors. The exposome concept developed by C Wild (2005) consists in the assessment of the combination of these exposures over life and is intended to complement the genome in epidemiological studies. It can be addressed through the identification of relevant biological markers using large scale methods such as metabolomics, adductomics, transcriptomics and other omics, in an integrated approach, but other methods assessing exposure are also relevant.

In addition to identifying exposures, one of the daunting challenges from a mechanistic point of view is to study the effect of a combination of stressors, for example the interaction between the thousands of chemical implicated in human contamination. Different approaches have been proposed to address the effect of chemical mixtures. One of them is to focus on the interaction of toxicological pathways as a first step. We have studied the interaction of ligands of the aryl hydrocarbon receptor (dioxin) with activators of the PXR and ER pathway (endosulfan) as well as with genotoxicants, using either large scale transcriptomics or targeted signalling studies. With large scale studies on the combination between dioxin and endosulfan, different types of effects were observed (additive, antagonistic and in very few cases, synergistic) (Ambolet-Camoit et al, submitted).

When endogenous metabolic pathways were studied, the combination displayed specific effects on endogenous metabolic pathways. In the case of the interaction between dioxin and etoposide, the activation of apoptosis elicited by the latter was prevented by dioxin (Ambolet-Camoit et al, Tox sci, 2010). In a more global approach, combination of chemicals could be tested on a large variety of toxicological pathways such as those assayed in the US Toxcast and Tox 21 programs.

Ultimately, the interaction between different types of stressors should be addressed. This will provide a mechanistic approach to the exposome rather than a purely descriptive one.

Prevention of diabetic risk through actions on the environment

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Identification of an Alzheimer’s disease-specific phenotype in blood platelets and development of a platelet biochip array

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Background: Alzheimer’s disease (AD) is a multifactorial neurodegenerative condition and is expected to be provoked by genetic and environmental factors. The best ante-mortem diagnostics available are neuropsychological assessments and brain imaging. However, these tests are prone to subjective decisions and not suitable for screening of huge numbers of suspects. Despite the discovery of a variety of potential markers, none of them is yet applied in clinical routine.

Objective: Consequently, alternative sample materials and minimally invasive diagnostic procedures are urgently needed. Based on the shared biological similarities of platelets and neurons, these peripheral cell fragments were used to characterize AD-specific biomarkers.

Design: Blood was collected from 62 patients with clinically suspected AD and 63 age/sex-matched controls. Their platelet proteomes were resolved in two independent discovery and verification phases using fluorescence 2-dimensional differential gel electrophoresis. AD-related proteins spots were identified after tryptic digestion by nanoflow liquid chromatography and MS/MS fragmentation. For high-throughput analysis we developed a novel multiplex platelet protein biochip to detect the revealed AD biomarkers from easy available platelet-rich plasma.

Results: Four proteins were highly significantly up-regulated in LOAD samples: monoamine-oxidase-B (MaoB), tropomyosin-1 (Tm1), apolipoprotein E4 (ApoE4), and glutathione S-transferase omega1 isoform A140 (GSTO1*A140). While both isoforms result from single nucleotide polymorphisms, the latter was highly predominant in APOE ε4–negative patients: genotyping revealed significantly more APOE ε4 carriers in the AD (66%) than in the control (11%) group and presence of exclusively two GSTO1*A140 alleles in non-APOE ε4 AD patients (n=20) relative to 38% in controls (30% in non-APOE ε4 controls) and 32% in APOE ε4–positive AD patients. Biochip analysis correctly identified 98% of all samples of the GSTO1*A140 and 100% of the APOE ε4 genotype by normalisation with either ERK2 or panApoE concentrations. Biochip quantification of Tm1 and MaoB (ERK2-normalised) also replicated the higher expression of these two proteins in AD patients relative to controls. An algorithm utilising these four biomarkers yielded the highest separation power for AD and control samples with a ROC AUC of 0.969 (95% CI=0.944-0.994).

Conclusion: This demonstrates the utility of this innovative multiplex device as reliable peripheral diagnostic tool to aid ante mortem AD diagnosis in a routine blood-based clinical screening.

27 September 2014 – Afternoon

SESSION X–THE FUTURE OF HEALTHCARE WITH OMICS

Clinical Bioinformatics: A paradigm change in Medicine

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There has always been interaction between the fields of medicine and biology, but never before has it been so strong. Recent developments in biological and medical sciences, i.e., the emergence of cost-effective, high-throughput, data-generating technologies, which are present in numerous areas from imaging to genomics, are likely to have a major impact in not too far a future on the daily practice of medicine. In less than a decade, it is highly likely that clinicians will be confronted with whole genome sequence data for a given patient, not to mention other types of data such as longitudinal epigenetic, RNA-Seq and protein expression data for a number of tissues and conditions as well as metagenome data. Clinical utilization of the ensuing data tsunami poses novel analytic, technological, educational and ethical challenges to clinicians and scientists. Clinical bioinformatics is devoted to fostering to the benefit of the patients clinical implementations of bioinformatic treatment of these various types of Big Data. These factors combined will contribute to a shift from evidence-based medicine to data-driven individualized or precision medicine, wherein “patients” will be seen as a whole, as a complex system rather than through the traditional organ-based medical paradigm, and thus radically change the way medicine is carried out.

Where’s the person in personalized medicine?

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The ‘personal’ aspects of personalized medicine are open to a number of interpretations. From one point of view medicine has always been personalized, but this claim exposes the differences between individualization and personalization. Categorization has also been an issue. From another point of view personalization involves respect for the autonomous patient, and this leads to issues about personalization in relation to diminished or developing capacity. In recent times, personalization has been associated with variation in the genome. The metaphor of tailoring has been used, but there are interesting differences between ‘bespoke’ tailoring and ‘made to measure’, which lead to questions about whether this is the appropriate metaphor. This is especially the case when personalization in relation to disease types and different stages in the life cycle is taken into account. Recently advances in epigenetics have added an extra dimension of complexity to the issues, as regards both causation, and personal and institutional responsibility.

The Synergy-COPD project: Modelling and simulation environment for systems medicine (Chronic Obstructive Pulmonary Disease -COPD- as a use case)

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Background - Chronic Obstructive Pulmonary Disease (COPD) has a major impact on healthcare. Heterogeneities in clinical manifestations and in disease progression are a relevant trait in COPD with impact on patient management and prognosis. It is hypothesized that COPD heterogeneity results from the interplay of mechanisms
governing three conceptually different phenomena: 1) pulmonary disease, 2) systemic effects of COPD and 3) co-morbidity clustering. **Objectives**—To assess the potential of systems medicine to better understand non-pulmonary determinants of COPD heterogeneity. To transfer acquired knowledge to healthcare enhancing subject-specific health risk assessment and stratification to improve management of chronic patients. **Method**—Underlying mechanisms of skeletal muscle dysfunction and of co-morbidity clustering in COPD patients were explored with strategies combining deterministic modelling and network medicine analyses using the Biobridge dataset. An independent data driven analysis of co-morbidity clustering examining associated genes and pathways was done (ICD9-CM data from Medicare, 13 million people). A targeted network analysis using the two studies: skeletal muscle dysfunction and co-morbidity clustering explored shared pathways between them. **Results**—(1) Evidence of abnormal regulation of pivotal skeletal muscle biological pathways and increased risk for co-morbidity clustering was observed in COPD; (2) shared abnormal pathway regulation between skeletal muscle dysfunction and co-morbidity clustering; and, (3) technological achievements of the projects were: (i) COPD Knowledge Base; (ii) novel modeling approaches; (iii) Simulation Environment; and, (iv) three layers of Clinical Decision Support Systems. **Conclusions**—The project demonstrated the high potential of a systems medicine approach to address COPD heterogeneity. Limiting factors for the project development were identified. They were relevant to shape strategies fostering 4P Medicine for chronic patients. The concept of Digital Health Framework and the proposed roadmap for its deployment constituted relevant project outcomes. **Keywords:** Chronic Diseases; Chronic Obstructive Pulmonary Disease; Exercise; Integrated Care; ICT; Systems Medicine; Predictive Medicine; Training

Time to reverse GWAS! Phenome-wide association studies
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Genome-wide association studies (GWAS) have been largely used the last 5 years to investigate and evaluate the associations between the variation of hundreds of thousands, to over a million, genotyped single nucleotide polymorphisms (SNPs) and single diseases/ outcomes or subphenotypes directly associated with chronic diseases risk factors (also called intermediate phenotypes or endophenotypes or health-related intermediate traits). Hundreds of SNPs have been associated with the risk of complex diseases, such as Cardiovascular diseases, type 2 diabetes and osteoporosis, as well as with intermediate phenotypes such as elevated circulating low density lipoprotein (LDL) cholesterol levels and glucose. However, even if the associations found by these studies have provided new leads toward a better understanding of the etiology and underlying biology of disease they have explained only a small proportion of the heritability of these traits. It is accepted today that GWAS present some limitations and suffer from important shortcomings. A common limitation of GWAS is the focus on a pre-defined and limited phenotypic domain such as the specific presence or absence of a single disease or the variability of one intermediate phenotype and neglects the field of phenomics which has also the recent past years proposed important network structures, giving thus a potential additional power to GWAS through the use of intermediate phenotypes that may more closely reflect a gene’s mechanism, as well as to the relationships between genetic variation and multiple diseases and endophenotypes (pleiotropy). A combined GWAS and phenomics strategy, known as phenotype-wide association studies (PheWAS), which is both, the reverse and the complement of the GWAS, would eliminate some of GWAS constraints and help for a better understanding of the pleiotropy of chronic diseases-related genes. This novel approach combines in fact both the exploration of phenotypic structure and genotypic variation and utilizes all available phenotypic information and all genetic variants in the estimation of associations between genotype and phenotype.

This approach changes the paradigm of phenotypic characterization and allows an exploratory research in both genomics and phenomics. The results can be used to discover novel relationships between SNPs, phenotypes, and network of phenotypes to foster hypothesis generation.

In the present study, we intended to determine, in a pre-planned two-step GWAS/PheWAS strategy, the pleiotropic effect of SNPs associated to Vascular Endothelial Growth Factor (VEGF). Our goal was to establish a synergy or antagonism between pathways involved in VEGF-related traits. Four polymorphisms (rs6921438, rs6416670, rs6993770, rs10738760) explaining ∼50% of VEGF heritability (estimated to be 60%) were identified by GWAS. These variants had significant effects on HDL, LDL, TNF-α, IL-6, E selectin and ICAM-1 plasma levels in healthy adults. Recent findings indicated that genetic variants of NOS3, CD14, MMP3 and IL4R are implicated in the determination of VEGF gene expression and plasma levels. Our PheWAS resulted to significant results indicating molecular links between VEGF and diseases biology and support the hypothesis that under physiological conditions there are complex biological relationships between different pathways (such as angiogenesis and inflammation), which are involved in the development of chronic diseases.

Recent developments in the Estonian Biobank and personal medicine program in Estonia
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Healthcare expenses are increasing in all developed countries, and as the average age and weight of the Estonian population is also increasing year by year, its expenses paid towards treating chronic diseases will follow suit in the coming years. In order to provide the necessary public healthcare over the next 10 years and beyond, it is not sufficient to simply adjust the current system. A paradigm shift is necessary. A new concept called *stratified medicine* or *personal healthcare* has taken root globally. It is based on using in addition to the presently used methods individual genomic variation obtained by genetic analysis and computational methods to predict and
prevent diseases and adverse drug reactions, as well as find optimal drug dosages. With the information and technology available today and the new knowledge, which is coming with great speed, such an approach makes it possible to establish significant results in regard to preventing or at least delaying common complex disease (cardiovascular and metabolic diseases, certain cancers, etc.). In addition to the aforementioned, it is necessary to increase the personal involvement of patients themselves through increased awareness of risks and more active health behavior (diet, physical activity, alcohol, tobacco, etc.).

Implementation of precision medicine in Estonia is feasible both in terms of scientific information and competence and in terms of IT infrastructure (EMR, e-Health, digital prescription, digital images, digital patient files, digital signature etc.) as well as financially, using EU Structural Funds for the period of 2014-2020, as the implementation of personal healthcare will require considerable expenses in the given years.

A huge leap in the field of genetics and a direct investment in the area was already made 10 years ago through the establishment of the Estonian Genome Center and the Estonian Biobank. Additionally, the e-Health database has been established and the digital prescription system has been applied and is currently working. Without such an infrastructure in place, Estonia would not even be in a position to talk about implementing genetic research for the benefit of public health. It is important to keep in mind that the EMORI survey carried out in Estonia also demonstrated the public’s strong support (75%) for genetic studies.

In conclusion, Estonia is probably one of the best places to start with the implementation of the personalized medicine and last not the least the Estonian government has taken this as one of its priorities for the coming years.

GENERAL DISCUSSION ON THE IMPLEMENTATION IN EUROPE OF SYSTEMS MEDICINE AND PHARMACOGENOMICS

- Angela Brand, Maastricht, Netherlands
  *Stratified medicine between personalized and systems medicine (Permed)*
- Charles Auffray, Lyon, France
  *The road map for implementation of Systems Medicine in Europe (Casym)*
- Magdalena Kalata, Brussels, Belgium (EDMA)
- Elisabetta Vaudano, IMI, Brussels, Belgium
- Christina Kyriakopoulou, EC, Brussels, Belgium
- EMA representatives
- Kirsten Steinhausen, Strasbourg (ESF)
- and all interested participants