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Poster abstracts

Poster abstracts:

AIPGENE

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http://www.aipgene.org/

SEVENTH FRAMEWORK PROGRAMME

AIPGENE: Augmenting PBGD expression in the liver as a Novel Gene therapy for Acute Intermittent Porphyria.

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Acute intermittent porphyria (AIP) is a rare genetic disease in which mutations in the porphobilinogen deaminase (PBGD) gene produce insufficient activity of a protein necessary for heme synthesis. This leads to an accumulation of toxic intermediates resulting in a wide variety of problems including acute, severe abdominal pains, psychiatric and neurological disorders, and muscular weakness. Acute porphyric attacks can be life-threatening and the long-term consequences include irreversible nerve damage, liver cancer and kidney failure. It is estimated that about 1 in 10,000 Europeans or people of European ancestry carries a mutation in one of the genes for acute porphyria. The therapies currently available do not prevent the symptoms or consequences of acute porphyric attacks. The only curative therapy is liver transplantation and thus, new curative options are clearly needed. In 2009, the European Medicines Agency granted Orphan Drug Designation to AAV5-AAT-PBGD for the treatment of AIP. AAV is a replication-incompetent virus that has been modified to deliver genes or genetic material into human tissues or cells. AAV5-AAT-PBGD acts by delivering the PBGD expression cassette directly into hepatocytes. In an animal model for AIP intravenous administration of AAV5-AAT-PBGD In heterozygous AIP patient that show 50% of the normal activity the additional PBGD activity will be sufficient to prevent the accumulation of toxic metabolites and thus, to prevent porphyric attacks. The aim of this project is the clinical development of the orphan drug AAV-AAT-PBGD for use to treat AIP. The project was performed in two different phases. In the first phase, a GMP-compliant process to produce sufficient AAV5-AAT-PBGD for clinical trials has been developed, and AIP patients have been followed up for a minimum of 6 months before entering the clinical trial. In the second phase, that has just started, the safety of AAV5-AAT-PBGD is being explored in a dose escalation clinical phase I trial, the efficacy of the treatment will be analysed.

Antisense oligonucleotide-mediated knockdown of TGF-β/myostatin type I receptors as a potential therapy for Duchenne and other muscular dystrophies

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Skeletal muscle fibrosis and impaired muscle regeneration are the major factors that contribute to progressive decline of muscle function in Duchenne Muscular Dystrophy (DMD) and other types of muscle dystrophies. Transforming growth factor- β (TGF-β) and myostatin are potent muscle growth inhibitors and involved in the regulation of muscle cell differentiation. Therefore, these inhibitors might be beneficial to improve regeneration and reduce fibrosis in the dystrophic muscle. We developed an efficient method to selectively inhibit the function of type I TGF-β receptors Acvr1b (ALK4) and Tgfbr1 (ALK5) based on antisense oligonucleotide (AON)-mediated exon skipping.

Our results show that both myostatin type I receptor ALK4 and myostatin/TGF-β type I receptor ALK5 can be efficiently downregulated *in vitro*. AON-mediated exon skipping in ALK4 and ALK5 receptors resulted in increased myogenic differentiation of C2C12 myoblasts. In addition, efficient AON-mediated knockdown of ALK4 and ALK5 was achieved *in vivo* after intramuscular and systemic injection in *mdx* mice, a DMD mouse model.

To summarize, our experiments suggest that this novel strategy of AON-mediated targeting of myostatin/TGF-β receptors may provide a therapy to selectively inhibit TGF-β, myostatin and activin signaling and improve muscle quality and function.

Further studies will investigate the short- and long-term effect of AON treatment in the DMD mouse model and other myopathic mouse models.

Arginine Therapy: A Novel Treatment for Children with Sickle Cell Disease and Vaso-occlusive Pain

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Background: Pain is the hallmark of sickle cell disease (SCD), a hemoglobinopathy that effects approximately 100,000 individuals in the United States, and millions worldwide. There are currently no effective therapies that target underlying mechanisms of pain, with symptomatic relief provided through analgesics and hydration as the only treatments available. The approach towards pain in SCD has changed little in decades, and negative attitudes towards SCD pain remain common in emergency departments (ED) across the nation. Novel therapies targeting SCD pain in the acute care setting will improve quality of life for patients, potentially decrease hospital length of stay (LOS), and improve knowledge and awareness of SCD in emergency medicine. Vaso-occlusive painful episodes (VOE) are the leading cause of hospitalizations and ED visits in SCD, and are associated with increased mortality. Low nitric oxide (NO) bioavailability contributes to vasculopathy in SCD. Since arginine is the obligate substrate for NO production, and we found that a dysregulation of the arginine metabolome in SCD is associated with pain and increased mortality, we hypothesized that arginine may be a beneficial treatment for children with SCD and VOE.

Objective: We conducted a randomized placebo-control trial (RCT) of arginine therapy in children with SCD hospitalized for pain to determine safety and efficacy of L-arginine treatment in this population.

Methods: Hospitalized SCD patients > 3 years diagnosed within 24 hours with VOE were eligible for participation. A standardized treatment and monitoring program for VOE was followed. 38 children with SCD hospitalized for 56 pain events at Children's Hospital & Research Center Oakland in years 2000-2008 were treated with parenteral/oral L-arginine (100 mg/kg TID) or placebo for 5 days or until discharge. Outcomes included total opioid use (mg/kg), pain scores and LOS.

Results: 56 independent pain events were evaluated. Mean age was 13.9±4 years (range 3.6-19 years), and 52% were female. Patients received intravenous (IV) or oral arginine (0.1 gm/kg TID, N=28) or placebo (N=28) for 5 days or until discharge from the hospital, whichever came first. Age and gender were equally distributed between treatment and placebo groups. Narcotic records for 2 patients (randomized to placebo arm) were incomplete and were not included in the opioid use analysis. 2 patients in the arginine arm were excluded after randomization as they received no parenteral opioids, an inclusion criteria. A significant reduction in total IV opioid use by 54% (1.9±2.0mg/kg vs. 4.1±4.1mg/kg, *p=0.02)* and lower pain scores at discharge (1.9±2.4 vs. 3.9±2.9, *p=0.01)* were observed in the arginine arm compared to placebo. There was no significant difference in LOS (4.1±01.8 vs. 4.8±2.5 days, *p=0.34*), yet a clinically relevant trend favored the arginine arm by 17 hours, and total opioid use correlated strongly to LOS (r=0.86, *p<0.0001)*. A larger increase in oxygen saturation at discharge was also noted in the arginine arm compared to placebo (*p=0.05*). No drug-related adverse events were observed**.**

Conclusion: This is the first RCT to demonstrate benefits of arginine therapy in children with SCD hospitalized for severe pain. Arginine therapy represents a novel and promising nutritional intervention for SCD. Use of parenteral arginine therapy should also be considered in the treatment of VOE in the ED setting prior to hospitalization, although further investigation is warranted. A reduction of opioid use by over 50% observed in this study is remarkable, together with lower pain scores at discharge and a trend towards a decreased LOS. This is the first successful intervention for sickle cell-related pain that targets the underlying mechanism of vaso-occlusion through a promising NO-based therapy. Arginine is a safe and inexpensive intervention with narcotic-sparing effects that should be considered as an adjunct to standard therapy for VOE. A larger-scale, multi-center trial is needed to confirm our observations.

Bioinformatic Disease Models for Huntington's Disease to Select Candidate Biomarkers and Drug Targets.

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Huntington's Disease (HD) is a progressive neurodegenerative disease resulting in impairments of motor, cognitive and psychiatric function. HD is a genetic, heritable disorder that results from expansion of CAG repeats in the HTT gene and thus an enlarged polyglutamine stretch in the resulting protein product. While mutation of the HTT gene is clearly the cause of disease, the mechanism by which this mutation leads to disease manifestation remains unclear. The mean age of symptomatic disease onset is 40 years of age with some patients experiencing early onset (< 20) and others unaffected until advanced age (>70). The disease progresses over a 15-20 year period after initial onset of symptoms. While diagnosis of HD is clearly made by sequencing of the HTT gene and may be determined at birth; biomarkers are needed to (a) predict the onset of symptoms, with an eye toward earlier intervention, (b) monitor disease progression to determine efficacy of potential drug treatments (i.e. serve as surrogate markers during drug trials) and (c) personalize therapy and treatment plans for individual patients (companion diagnostics). Ariadne Diagnostics has utilized a bioinformatic approach to scan the existing literature and integrate data regarding HD-related genes, proteins and protein-protein interactions. This information was utilized to identify HD-related cellular processes and molecular pathways which were manually curated to remove less relevant hits and expand those with the greatest significance. These initial disease models can be probed with targeted experimental "omics" data using a proprietary sub-network enrichment analysis (SNEA) to refine the model and identify candidate biomarkers and drug targets. In one example, we have utilized gene expression data derived from a cellular model (PC12 cells) of HD in which mutant HTT expression was induced and expression patterns were determined before (day 1) and after (day 5) significant protein aggregation occurred (van Roon-Mom, *et al.*, BMC Molecular Biology 2008, 9:8; GSE10581). This model identified specific molecular pathways that may be induced in early (prodromal) HD and at disease onset. In turn, key upstream-regulators and downstream-products of these pathways can be suggested as potential drug targets for maintenance in the prodormal state and as biomarkers for disease progression. In another example, we analyzed publicly available data (Borovecki, *et al.* (2005) *Proc Natl Acad Sci USA*. 102(31):11023-8; GSE1751) comparing gene expression levels for 12 symptomatic and 5 presymptomatic Huntington's disease patients versus 14 healthy controls. Again this data was used to identify biomarkers for the transition to symptomatic disease. These data sets can be used separately to refine and build specific models, but may also be combined to select biomarkers with the highest likelihood of success in following disease progression.

Canadian Values, Attitudes and Willingness to Pay for Expensive Medications for Rare Diseases.

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Purpose: Personalized medicines, particularly those for rare or orphan diseases, is an area of rapid development often associated with much higher prices than those for common diseases. The potential financial burden and opportunity cost of treating patients with rare diseases is therefore significant. As government health care budgets are limited, it is important to understand whether Canadians would altruistically prioritize a rare condition over the efficiency of using resources to benefit more people. The objective of this study was to determine how Canadian society values the treatment of rare over common diseases, willingness to accept the opportunity costs associated with funding rare diseases, and maximum willingness to pay (WTP).

Methods: A cross-sectional survey of the Canadian public was employed using a web based questionnaire. Respondents chose to fund treatment and allocate limited resources to either a rare or common disease, and preferences were elicited under both equal and higher rare disease treatment cost. Participants also rated five statements regarding rare disease equity attitudes. WTP was determined using a variation of the payment card method where respondents were presented either high or low initial price ranges. Respondents were also randomized to either high or low drug treatment efficacy.

Results: Data on 100 pilot respondents indicate strong agreement with the statements pertaining to rare disease equity. 57% of participants strongly agreed and 19% agreed with the statement "Everyone should have equal access to health care regardless of cost". When treatment cost was equal, 37% of subjects chose to treat the common disease, 35% the rare disease and 28% were indifferent. When the rare disease was more expensive to treat, the results were 23%, 53% and 24% respectively. WTP for high efficacy and a lower payment-card range ranged from $2500 to over $1 million, with an average of $238,000. For the lower efficacy scenario, WTP averaged $99,000 when randomized to lower payment ranges and $173,447 in the higher ranges.

Conclusion: Although Canadians feel positively towards statements pertaining to rare disease equity, preliminary data indicates no preference for rarity at the expense of common conditions. Additionally, the WTP varied depending on the treatment efficacy and the price ranges respondents were initially shown. The current costs for rare disease treatments, which are as high as $600,000 annually per patient, appear to exceed the societal WTP, particularly given the low or unknown efficacy associated with these treatments.

Cellular Approaches for Rare Pulmonary Diseases (CARPuD)

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In rare pulmonary diseases as α1-antitrypsin deficiency, cystic fibrosis, and surfactant deficiencies, stem cell-based approaches open new avenues for exploration of pathomechanisms or therapeutic targets, and for the evaluation of novel therapies. The generation of induced pluripotent stem cells (iPSCs) facilitates the generation of disease- and patient-specific stem cell lines. iPSCs can be easily grown in large quantities and genetically engineered. These cells allow for the first time correction of genetic defects, followed by in vitro differentiation into the desired pulmonary cell derivates and re-transplantation of the autologous cells for replacement of the affected cell type.

Based on our achievements of the first funding period, we now aim to extend the establishment of patient-specific human (h)iPSCs, to further improve and scale-up established differentiation protocols and to adapt these protocols from murine (m) iPSCs to hiPSCs. Furthermore, the resulting cells will be applied for advanced studies on cellular disease mechanisms and development of improved (stem) cell-based transplantation strategies for the respective lung diseases. In close collaboration with our clinical partners of the future German Lung Center, we will establish a patients' fibroblast bank to enable the generation of a variety of patient-specific stem cells. We expect that these joint activities will lead to first pre-clinical and clinical phase-1 studies in a third funding period with the perspective to be transferred to general clinical practice in the near future.

Clinical and immunological presentation of novel mutations in *GATA2.*

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Abstract

Introduction: *GATA2* autosomal dominant mutations have been described in patients suffering profound monocytopenia, dencritic cell, B and NK lymphoid deficiency with predisposition to nontubercular mycobacterial infection (Mono-MAC syndrome) and to myelodysplastic transformation. A recent study proposes allogenic stem cell transplantation as an effective therapeutic approach for *GATA2* mutation

Patients: Here we present two patients with novel mutations in *GATA2* gene, both with hypogammaglobulinemia, monocytopenia and absence of peripheral B and NK cells. Dendritic cells were also investigated showing the lack of lineages.

-The first patient, an 11 year-old girl previously healthy was admitted at the intensive care unit with disseminated varicella-zoster virus (VZV) infection and multiple organ failure. From 11 to 16 years-old, the patient stayed asymptomatic suffering only two lower respiratory infections. At present, the patient is asymptomatic and is being treated monthly with IVIG infusions and regularly controlled by the haematologist.

-The second patient corresponds with a 20 year-old man with lymphoedema and lymph nodes agenesia since the age of 3-years old. The patient suffered multiple and recurrent episodes of erysipelas and in the last 2 years started to suffer respiratory tract infections pneumonias and esophageal candidiasis.

During the last hospital admission laboratory tests showed pancytopenia (profound lymphopenia and pancytopenia) besides granulomatous lung and bone marrow lesions. Bone marrow aspiration analysis showed myeloid hyperplasia. Currently the patient is hospitalized for daily mild fever and profuse sweating and responding to empiric therapy for Mycobacteria.

Molecular study of *GATA2* gene revealed two novel mutations. In patient 1, the insertion of two nucleotids (AC) in the begining of exon 6 leads a frameshift mutation, (c.1156-1157insAC) leading an early STOP codon (p.Leu386HisfsX2) and lacking the nuclear localization signal domain from the protein. The parents were investigated and no mutations were found, indicating that the insertion of two nucleotides in this patient is *de novo* mutation.

In patient 2, the change at position 988 in the exon 5 (c.988C>T) leading also and STOP codon in exon 5 (p.Arg330X) located in the protein domain “Zinc finger domain 2”. The result is a truncated protein of 329 aminoacids (the native protein has 480 aminoacids), which generate a non-functional protein, implying a pathologic effect.

Conclusions: Here we describe the clinical and immunological presentation of two patients with novel and different mutations both leading early STOP codons in *GATA2*: 1- The insertion of two nucleotids 1156-1157insAC lacking the nuclear localization signal domain and 2- A change of nucleotid 988C>T disrupting “Zinc finger domain 2”. These cases differ in their clinical presentation and evolution. Treatment recommendations are under discussion in GATA2-deficiency patients with open questions as if to treat with allogenic stem cell transplantation in asymptomatic patients.

Cysteine Quantity Correction in CADASIL; modification of the NOTCH3 protein using antisense oligonucleotides.

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Introduction CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy) is the most common monogenetic cause of ischemic stroke and vascular dementia. CADASIL is caused by highly stereotyped missense mutations in the *NOTCH3* gene, which invariably lead to the gain or loss of a cysteine residue in one of the epidermal growth factor-like (EGF) domains of the NOTCH3 protein. This causes disrupted disulphide bridge formation and toxic NOTCH3 aggregation. We have developed a potential therapeutic strategy for CADASIL aimed at preventing NOTCH3 aggregate formation (Cysteine Quantity Correction). In this approach, antisense oligonucleotides (AON) are used to skip targeted exons from the *NOTCH3* pre- mRNA, in order to restore the correct number of cysteines in the mutated EGF domain.

Methods *In silico:* protein prediction programs were used to select exons that could be skipped to restore 6 cysteines in the mutated EGF domain of the NOTCH3 protein. *In vitro:* AONs targeting *NOTCH3* were designed and transfected in control fibroblasts and subsequently in patient derived vascular smooth muscle cells. Exon skipping was analyzed by RT-PCR and sequencing analysis.

Results Skipping of *NOTCH3* exons 4 and 5 targets up to 70% of CADASIL mutations, and is predicted to result in a modified NOTCH3 protein with an EGF fusion domain with normal disulphide bridge formation. Using a combination of 3 AONs, exon 4-5 skipping is efficient and robust *in vitro*.

Conclusions *NOTCH3* exon 4-5 skipping results in a theoretically favorable NOTCH3 protein *in silico* and is efficient *in vitro*. *In vivo* experiments in animal models are currently being performed to determine the possible therapeutic effect of this *NOTCH3* exon skipping strategy for CADASIL.

Title:
Data Analysis Pipelines at CNAG

Authors:
CNAG Bioinformatics Groups

Abstract:
The CNAG's mission is to carry out large-scale projects in genome analysis in collaboration with the Spanish, European and International research communities. CNAG is actively involved in the Chronic Lymphatic Leukemia, Prostate Cancer and Ewing Sarcoma projects from the International Cancer Genome Consortium. Furthermore, CNAG participates in FP7 projects such as ESGI, GEUVADIS, BBMRI, BLUEPRINT, SYBARIS, AirPROM, IBDCHARACTER or RDCONNECT. The integrated CNAG infrastructure is one of the largest in Europe. Over 600 Gbases/day can be generated with the thirteen second generation sequencers, supported by an extensive informatics infrastructure. CNAG is constantly seeking to improve and optimize data analysis to provide new applications and cope with the increasing amounts of data being generated. Therefore, research on faster cutting edge software and development of original and efficient state-of-the-art data analysis pipelines and file formats are central activities at CNAG.

DEM-CHILD - A Treatment-Oriented Research Project of NCL Disorders as a Major Cause of Dementia in Childhood

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The DEM-CHILD project focuses on the main cause for childhood dementia in Europe, the neuronal ceroid lipofuscinoses (NCLs). The NCLs are neurodegenerative diseases characterized by dementia, blindness, epilepsy and physical decline leading to an early death of the patients. Since no cure is currently available, these disorders represent a serious social, medical, and economic challenge.

To date, nine NCL genes have been characterized. There is evidence suggesting that further gene loci remain to be identified. NCLs are under-diagnosed in many countries around the world as there is an overall lack of research, early diagnosis, treatment and expert availability. Furthermore, due to their broad genetic heterogeneity it is difficult to collect large numbers of genetically similar patients. As such, large therapeutic studies required for advances in treatment are difficult to intiate.

The DEM-CHILD project will combine the expertise of (i) recognized European research teams with (ii) high-technology SMEs, and will (iii) collaborate with Indian experts on the following objectives: (1) High-technology SMEs will develop innovative cost- and time-effective testing and screening methods for all NCLs in order to ensure early diagnosis and thereby prevention; (2) DEM-CHILD will collect the world's largest, clinically and genetically best characterised set of NCL patients in order to study disease prevalence and precisely describe the natural history of the NCLs leading to the development of an evaluation tool for experimental therapy studies; (3) Novel biomarkers and modifiers of NCL will be identified to support the development of innovative therapies; (4) Focussing on the development of therapies for NCL disease caused by mutations in intracellular transmembrane proteins, two complementary therapeutic strategies will be used and compared in eye and brain of mouse models: a) viral-mediated gene transfer and b) neural stem cell-mediated delivery of neuroprotective factors.

The DEM-CHILD project has received funding (3 Mio. Euro for three years) from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 281234.

Developing innovative therapies for mitochondrial disease

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Collectively, mitochondrial diseases represent a highly significant health burden (conservative estimate for the prevalence of all mitochondrial diseases > 12:100.000). Mitochondria are present in all cells except for erythrocytes. Tissues with a high energy demand like brain, the eyes, heart and skeletal muscle are often affected. About half of the patients presenting in childhood die before the age of 10 years illustrating the urgent need for the development of effective treatments.

Treatment development strategy
Several cell biological consequences of mitochondrial dysfunction can be observed in primary cells of patients with mitochondrial disease. These consequences include: i) increased production of reactive oxygen species, ii) decreased mitochondrial membrane potential, iii) altered redox state, iv) alterations in the mitochondrial network formation, v) altered calcium signaling and vi) lower ATP production. These alterations are not only entrance points for therapy, but can also be used as readouts for the effects of compounds on cellular well-being.

From hit to lead compound to human drug
Of the different hit compounds detected in earlier studies one has been selected to enter the lead optimization phase. For this compound preliminary data demonstrated efficacy on muscle strength and survival in a highly representative mouse model. This “hit” compound is in the process of being optimized on potency, physicochemical and metabolic properties with the goal to arrive at a basket of at least four lead compounds with desired potency and ADMET properties. For this unique *in vitro* assays and *in vivo* disease models are used. Administration of these compounds to patient-derived cells should at least restore all of the above mentioned abnormalities seen as consequences of mitochondrial disease. Subsequently, a strong academic-industry consortium will perform toxicological, formulation and CMC activities with the goal to generate a compound-based drug for oral use in humans.

Natural History
Since mitochondrial disorders have a heterogeneous and unpredictable disease course, which is often oscillating, the best method to measure the effect of treatment is to compare the patient with its own natural history. In our centre, we are running several natural history studies in adults and children, using a multidimensional questionnaire, physical examination and quality of life. The most extensive study involves patients with a specific mutation in the mitochondrial DNA (m.3243A>G). At this moment, 125 patients with this mutation are followed yearly using a validated quantitative clinical scoring system. Only with the information from this study, we will be able to draw conclusions on the effect of medication in these patients with an unpredictable disease course.

Outcome measures
Every experiment, including clinical trials, is only as reliable as its endpoints. Since there is not much information about the reliability and validity of outcome measures in patients with mitochondrial disorders, we are currently testing multiple systematically selected instruments in children with mitochondrial disorders.

Future perspectives
We expect the first in man studies to be executed early 2015.

This work was sponsored by ZonMW

Dose-Escalation Trial of Digitoxin for the Treatment of Airway Inflammation in Cystic Fibrosis

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Cystic Fibrosis (CF) is one of the most lethal autosomal recessive, inherited orphan diseases in the world. There are less than 30,000 individuals with CF in the USA, making this disease rare and classified as an orphan disease. The lifetime morbidity is immense and the cost of new, personalized, genotype-specific therapies astronomic. CF results from the inheritance of two mutations in the CFTR gene which encodes a cyclic AMP-regulated chloride channel. Common to most cases of CF is an inflammatory phenotype in the lung, making the airways vulnerable to bacterial colonization, infection, and destruction. Airways inflammation in CF is predominantly neutrophilic and complicates airway clearance therapies through cellular debris, excessive DNA, excessive and viscous mucous, and high concentrations of Il-8 and related cytokines liberated along the NFkB signaling pathway. Inadequate inflammatory and infectious therapies allow airways obstruction, destruction, and ultimately death.

We undertook a proteomic and genomic approach to screen CF cell lines for genes and proteins in the inflammatory pathways that were overactive compared with nonCF cells. The central role of TNFalpha/Tradd in upregulating Il-8 and attracting neutrophils and macrophages was confirmed. Screening of CF cell line responses to cardiac glycosides identified one well-known drug—digitoxin—as most effective and potent in reducing Il-8 secretion. Importantly, digitoxin had similar overlapping beneficial effects on genes as did treatment by gene therapy to restore normal CFTR. Digitoxin is no longer approved by the FDA in the USA, so an Investigator IND was obtained to study the medication in CF.

The “Phase II Study of Digitoxin to Treat CF” is a randomized, double blind, placebo-controlled, repeat dosing, proof-of-concept trial in which we will evaluate the effects of 28 days of digitoxin administration on inflammatory markers in induced sputum obtained from subjects with mild to moderate CF lung disease. CF patients who are 18 to 45 years old may be eligible for screening. Our primary objective is to measure the effects of digitoxin on IL-8 and neutrophil counts in induced sputum in stable CF patients. Secondary objectives are 1) to measure the pharmacokinetics of digitoxin in serum in stable CF patients, 2) to measure safety indices, including ECG changes and sputum microbiology, in stable CF patients, 3) to measure the effect of digitoxin on gene expression in nasal epithelial cells of stable CF patients and 4) to measure quality of life scores using the CFQ-R©.

This is a single site study that is conducted in the Johns Hopkins University GCRC of the Institute for Clinical and Translational Research (a CTSA funded CRU). Each subject will participate for up to 10 weeks. Sixteen evaluable subjects will be treated with digitoxin (0.05 mg or 0.1 mg daily), and 8 evaluable subjects will be treated with placebo. Digitoxin is supplied by Merck KGaA, Dharmstadt. Placebo is supplied by the Johns Hopkins Investigative Pharmacy and drug and placebo are re-encapsulated for blinding. As of February 20, 2013 there have been no serious adverse events attributable to study drug and after the initial interim analysis, the study Data Safety Monitoring Board approved escalation to the higher dose of digitoxin.

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DRUGS4RARE: Drug Discovery Platform for Rare Diseases

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We have developed a platform for Drug Discovery for Rare Diseases, Drugs4Rare, with three main actions: a) to gather information on the chemical structures, published mechanisms of action (including biological targets), and orphan diseases, associated to chemical compounds of therapeutic interest; b) to identify the potential protein targets of each of those compounds by means of state-of-the-art in silico target profiling approaches; and c) to validate experimentally the highest affinity predicted chemico-biological interactions.

The results of these three consecutive actions are deployed in an integrated chemical biology annotated Drugs4Rare database, which contains key virtual and experimental information in the drug discovery process for new orphan drugs for rare diseases, or for repurposing of therapeutically active compounds towards rare diseases.

In order to generate the Drugs4Rare Drug Discovery platform, we have analyzed 49 chemical compounds which have been approved in Europe for the treatment of certain rare diseases. After gathering information about these compounds and associated rare diseases from the Orphanet database (www.orpha.net), we analyzed the information on their chemical structures, biological targets, and mechanisms of action in public (Pubchem, ChEMBL) and private access (SciFinder, Integrity) databases. Then we used the virtual polypharmacology predictive platform developed in the Chemogenomics Laboratory at IMIM, which allowed us to generate a profiling of the 49 compounds in front of around 4500 biological targets. Analysis and prioritization of this virtual chemical biology matrix, based on the commercial availability of the selected compounds, and on the availability of biochemical assays for the highest affinity predictions, led us to the selection of three compounds, for which experimental validation of new biological targets was determined.

We are currently expanding this initial “proof of concept” of our Drugs4Rare Drug Discovery Platform to additional compounds under study for the treatment of rare diseases, from the Orphanet or other databases, as well as to other compounds of therapeutic interest, that could eventually be reprofiled for the treatment or rare diseases.

ENERCA 3: EUROPEAN REFERENCE NETWORK OF EXPERT CENTERS IN RARE ANAEMIAS

Maria del Mar Mañu Pereira, Laura Olaya Costa and Joan Lluis Vives Corrons (On behalf of ENERCA consortium)

ABSTRACT

Background
The European Network for Rare and Congenital Anaemias (ENERCA), a project co-funded by the European Commission (Public Health and Consumer Protection Directorate), was set up in 2002 to help medical practitioners and patients deal with rare anaemias (RA) by improving the public health service in this regard. .The main goal is the improvement of the health services for RA offered across Europe assuring the same level of access for both health professionals and patients independently of their country of practise or origin.
Objective
The objectives of ENERCA 3 Project includes: 1) the consolidation of the European Reference Network (ERN) of Centres of Expertise (CEs) in RA for cross border healthcare 2) the promotion of harmonized procedures for diagnosis and treatment of RA including the preparation of a comprehensive catalogue for the External Quality Assessment Schemes (EQAS) in diagnostic tests for RA 3) the provision of a tool for epidemiological surveillance of RA in Europe. 4) Improvement of continuous medical education in order to insure the provision of the highest quality services for patients with RA. 5) Increase of patients and public awareness about RA. 6) The promotion of research and cooperation between experts in RA by providing support to the development of strategies and mechanisms necessary for a continuous exchange of information and 7) The promotion and recognition of the ENERCA web www.enerca.org
Methods
ENERCA has been undertaken by 48 partners covering the majority of MS. The methods have been mainly focused on the establishment of the consensus criteria necessary to become an Expert Centre in the ERN for RA, the analysis of the legal framework existing between MS for patients and blood samples referral with the aim of overcome the administrative barriers created by the different national rules and laws, the establishment of close links between experts in order to gather epidemiological data and create a Epidemiological European Registry for patients with Rare Anaemias, elaboration of standardized guidelines for clinical practice in RA, and the development of educational and training activities for improving the knowledge on RA. Continuous medical education has been assured by the celebration of courses, workshops and symposiums on RA.
Results
The main results of this phase of ENERCA are: 1)The recommendations for CEs (ENERCA White Book) in the field of RA.ENERCA recommendations for CEs provide stakeholders involved in the national Plans for RD and EC with a practical material and specific methodology necessary for moving towards the establishment of consensus recommendations for CEs to become nodes within a future Rare Diseases European Reference Network (RD ERN) in RAs. 2) The analysis of the available EQAs for RA laboratory procedures have allowed the establishment of an inventory of core laboratory tests used across Europe for RA diagnosis, a catalogue of EQAs and EQAs' providers. 3) Continuous Medical Education (CME) to health professionals through the organization of three European training courses on specific topics regarding diagnosis and management of RA, trough the ENERCA symposiums and ENERCA website, an open door for both health professionals, including health authorities, and patients to ENERCA. 4) ENERCA guidelines have been published on different topics involving the diagnosis and clinical management of patients affected by haemoglobinopathies.
Conclusions
The achievement of the Project's objectives and outcomes will contribute to the improvement of health and quality of life of patients with RA by increasing the efficacy of patient's diagnosis, treatment and follow up. Further efforts have to be done mainly in the promotion of the recognition of CEs at national level, a cornerstone in the process of official establishment of ERN, and promotion of new e-Health tools based on the creation of electronic health records (EHR) for epidemiological surveillance, and on-line platforms for e-learning and tele expertise and telemedicine services across Europe. These are the main goals of the new project, e-ENERCA which is currently under negotiation with the EAHC.

E-Rare project:
Congenital neutropenia with ELA2 mutations (ELA2-CN): Evaluation of genetic co-factors and molecular pathways with respect to the heterogeneity on phenotype
C. Zeidler, P. Coffer, H. Tamary, I. Touw, D. Yilmaz-Karapinar, K. Welte

Introduction
Congenital neutropenia patients with ELA2 mutations (ELA2-CN) and cyclic neutropenia patients harbouring ELA2 mutations (ELA2-CyN) represent approx. 60% of inherited neutropenia patients in Europe and North America. Other genetic defects causing CN like HAX1-, G6PC3-mutations have been identified in minor cohorts of consanguineous families in Europe only. Although both, the phenotype of CN and CyN are related to identical ELA2-mutations, the clinical course differs significantly: ELA2-CN patients have a high risk for secondary leukemias, require higher G-CSF doses and develop G-CSF receptor mutations, whereas ELA2-CyN respond to lower G-CSF doses without malignant transformation showing the typical cycling of neutrophil counts throughout their life. It is still unknown how ELA2 mutations contribute to these different clinical phenotypes. We hypothesized the existence of modifying molecular defects and mutator genes in patients with ELA2 mutations discriminating between the two major phenotypes (CN and CyN).
The E-rare network consists of projects related to the collection of long-term clinical data and biomaterial (Registry-biobank projects) and basic research within four countries (GER, ISR, NED, TUR). Within the network we exchange information and biomaterial on all patients suffering from ELA2-CN or ELA2-CyN.

Results
Genotype-phenotype correlation
We have collected clinical data on 91 ELA2-CN and 32 ELA2-CyN patients for genotype-phenotype correlation within our network.

Modifying genes
In samples from patients with ELA2-CN who developed leukemia we have identified Runx1- and ASXL1 mutations in the leukemic cell sample of these patients.

Molecular pathways
We found significantly elevated phospho-STAT5 levels in CN-ELA2 patients compared to CyN-ELA2 patients and healthy individuals. We could show that hyperactivated STAT5 induces ubiquitination and degradation of LEF-1 protein. We were also able to show that bortezomib is able to inhibit LEF1 degradation. We plan to study other proteins involved in STAT5 activation, e.g. cbl, SHIP, SOCS3.

We also analysed the effects of different ELA2 mutations on Nampt/NAD+/SIRT activation and deacetylation of p53 and FOXO3a and found substantial deacetylation and subsequent deactivation of these proteins.

ELA2 induced pathomechanisms
We compared the effects of ELA2 gene mutations, which are specific for CN, with ELA2 mutations common for both, CN and CyN patients, on the induction of UPR, (BiP and ATF6 target genes). We found elevated expression levels of ATF6 and ATF6 target genes in cells transduced with ELA2-CN mutations, in comparison to ELA2-CN/CyN mutations.

Epigenetic alterations
To identify epigenetic alterations or passenger mutations in addition to ELA2 mutations we performed whole genome sequencing a) in one family with two patients with congenital neutropenia (Father, Mother, Sister, two affected CN patients) and b) in one family of a patient with cyclic neutropenia (Father, mother, brother, Cyclic patient). So far we have detected sporadic nonsense mutations in the genes GRM1 and ACAP2 in one CN patient and an inherited mutation in TNFRSF1A in a cyclic neutropenia patient. The validation of these genes are in progress.

Conclusion
Our E-Rare network on Congenital neutropenia with ELA2 mutations (ELA2-CN and ELA2-CyN) demonstrates a successful network-cooperation to improve the knowledge on a rare disease by sharing clinical data and samples. In addition, we merged our data with data from North America and recently submitted a manuscript on 307 patients with ELA2-CN or ELA2-CyN describing genotype-phenotype relationships and the prediction of clinical outcomes based on the genotype. To improve statistic evidence there is clearly a need for transatlantic cooperation in rare genetic disorders.

International Rare Diseases Research Consortium Conference 2013

Poster Abstract

Title: “EURO-CDG: A European research network for a systematic approach to Congenital Disorders of Glycosylation and related diseases"

Authors: Gert Matthijs on behalf of the EURO-CDG consortium

Congenital Disorders of Glycosylation (CDG) are a growing group of rare inborn errors of metabolism. Patients present with an extremely variable and complex phenotype, and more than 40 causes of CDG have been defined genetically. Recent discoveries suggest that any defect that disturbs the function and organization of the intracellular compartments may lead to an abnormal glycosylation and thus cause CDG.

The work covers 3 different aspects:

1. Increasing the speed of diagnosis and disease identification will allow us to feed novel data into the biochemical, glycobiology and cell biological studies. This will be done by a systematic analysis of candidate genes, and by novel genetic approaches like exome sequencing.

2. The results will naturally be translated into the development of cellular and animal models that are essential to study the pathogenesis and an important step towards the development of therapies. A thorough analysis of the large group of PMM2-CDG (CDG-Ia) patients for modifier genes should help to explain phenotypical variability and allow to pinpoint pathways that may be targets for supportive therapy.

3. Therapy and cure are difficult to tackle in the case of CDG. For instance, the defects are situated in intracellular compartments that cannot (yet) be targeted by recombinant enzyme treatment. Also, the disease is largely non-progressive. Still, there is room for supportive therapies that would improve the patients’ life.

Research into CDG in Europe has been steadily growing over the past 10 to 15 years, thanks to 2 consecutive, collaborative initiatives that were funded by the European Commission, namely EUROGLYCAN and EUROGLYCANET. The backbone of these projects was a shared database and sample repository. The success was derived from an intense collaboration between clinical and fundamental researchers and from the establishment of national referral centres for CDG.

The current project wants to build on these achievements. The 6 groups that work together on this project have all been involved in the unravelling of different types of CDG and are committed to work together to further increase the pace towards disease identification, the study of pathophysiology and the development of therapies, for the benefit of the patients and their families.

Contact: www.euroglycanet.org

International Rare Diseases Research Consortium Conference 2013

Poster Abstract

Title: “EuroGentest: Harmonization, validation and standardization in genetic testing."

Authors: Valerie N. De Groote, Gert Matthijs on behalf of the EuroGentest partners

EuroGentest is a European network financially supported by the European Commission (FP7) aiming to insure that all aspects of genetic testing remain of high quality thereby providing **accurate and reliable results for the benefit of the patients**.

High quality genetic testing is a process starting from the correct indication for the test, a fair way to fund testing and the prioritization needed for that. It also includes pre-test counselling and consenting to the test, taking and sending the sample with adequate clinical information, a correctly performed test with an adequate interpreting of the result to the referring clinicians, post-test counselling and the other post-test actions like informing relatives and organizing possible follow up. Thus, EuroGentest strongly aims at **improving the quality of all genetic services** associated with genetic testing, across Europe, and at all the stages in this process. **Workshops** on quality management are organized to aid the laboratories in working towards accreditation. **Clinical Utility Gene Cards** (disease-specific guidelines regarding the clinical utility of genetic testing) are set-up to provide guidance to clinicians, geneticists, referrers, service providers and payers.

Genetic testing for rare diseases is an interactive process involving the patient and his/her family, the genetic laboratory and the referring clinician or clinical geneticist. For all genetic tests, accurate results and interpretation are essential as the results have huge consequences for the patient and their family. The patients will benefit by the improvement of the analytical and clinical quality and validity of the testing, and from improved trans-border services and information.

EuroGentest has generated the **patient leaflets** where general information on genetic testing is available in a comprehensive way in more than 27 languages.

EuroGentest **members** are experts from all over Europe (Belgium, Ireland, UK, France, Spain, The Netherlands, Switzerland, Finland, Sweden, Portugal, and Czech Republic). They contribute to the establishment of **guidelines and recommendations**, in a concordant way and in collaboration with the professional organisations.

One of the aims of the project is to create a European association of genetic diagnostic centers that will guarantee the future of the network. The European diagnostics industry will benefit through a faster access of innovations to the market through the validation for diagnostic use. It will enable countries and regions with less developed health care infrastructure to develop standards for best practice of provision of clinical genetic service.

Contact: www.eurogentest.org

EUROmediCAT: SAFETY OF MEDICATION USE IN PREGNANCY IN RELATION TO RISK OF CONGENITAL MALFORMATIONS

H Dolk (1), M Loane (1), MK Bakker (2), LTW de Jong-van den Berg (3), & EUROmediCAT Working Group
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Objectives: Many women need to use medication in pregnancy, while the risk for the developing fetus of many of these medications has not been established. Surveillance of possible adverse effects requires the use of large databases to assess rare exposures and rare outcomes. In EUROmediCAT, a Framework 7 funded study 2011-2015, we aim to build a European system for reproductive safety evaluation based on congenital anomaly (CA) registers in Europe (EUROCAT) combined with existing health care databases. We focus in particular on prescribed medications for chronic diseases.

Methods: The project involves 14 EUROCAT CA registries which record medication exposure during the first trimester of pregnancy, 6 healthcare databases (including primary care databases and prescription databases) of which 5 are being linked to CA registries, and a cohort study of diabetic pregnant women linked to 9 of the CA registries. A variety of study designs are being tested and applied: a) signal detection methods detecting unusual associations between specific CA and specific medications b) case-malformed control and cohort studies of four drug groups: antiepileptic drugs, insulin analogs, antidepressants, and antiasthmatics c) drug utilization studies of the four drug groups to assess the frequency of their use by women in the population before, during and after pregnancy d) internet research methods to assess the use of the internet for medication purchase and safety information.

Results: The project dataset from CA registers now contains 81,770 congenital anomaly registrations, covering a total population of 6.4 million births 1995-2010. Linkages with the healthcare databases have been performed, but for data protection reasons a distributed database design is needed where all linked datasets are kept in the country of origin. Common software is operational to support the linked dataset analysis and diabetic cohort study, building on the EUROCAT Data Management Programme. A common protocol has been implemented in all healthcare databases for the drug utilization studies, which allows country differences in the frequency and type of medication use in pregnancy to be described.

Discussion and Conclusions: EUROmediCAT is in a unique position to conduct population-based postmarketing surveillance of drug safety in pregnancy in Europe, based on the EUROCAT network. A variety of methodological approaches are needed for effective pharmacovigilance, and must be brought together to build a sustainable and effective system for Europe which will give women and clinicians the information they need to weigh the risks and benefits of different medication options.

European Multidisciplinary Initiative on Neuroacanthocytosis

Neuroacanthocytosis Syndromes

The rare conditions collectively labelled as neuroacanthocytosis (NA) seriously disable young adults and place a heavy burden on them and their families. NA syndromes affect about 1 in 3 million individuals and present a variety of emotional, cognitive and movement disorders. They share considerable similarities with Huntington´s disease (HD). As in HD, the basal ganglia are preferentially affected by neurodegeneration but in contrast to HD a key to the understanding of underlying mechanisms may be found in easily accessible peripheral cells.

It is characteristic for NA that deformed erythrocytes with thorny protrusions are found in the patients´ blood. Although not detectable in every single case, these cells are the origin of the term neuroacanthocytosis, denoting the association of neurological findings and acanthocytes ("akantha": Greek for thorn).

NA syndromes may be divided into those with lipid abnormalities and peripheral nervous system involvement (such as abetalipoproteinemia) and into the "core" NA syndromes with central nervous system involvement, including basal ganglia degeneration. Currently, the genetic basis is known for four of these conditions: McLeod syndrome (MLS, X-linked), chorea-acanthocytosis (ChAc, autosomal recessive), Huntington´s disease-like 2 (HDL2, autosomal dominant) and pantothenate kinase associated neurodegeneration (PKAN, autosomal recessive). Responsible genes are the Kell protein associated XK in MLS, VPS13A (vacuolar protein sorting 13 A, chorein) in ChAc, JPH3 (junctophilin 3) in HDL2 and PANK2 (pantothenate kinase 2) in PKAN. The recognition of PKAN as part of the NA spectrum creates a link to the NBIA group of syndromes ("neurodegeneration with brain iron accumulation"). NBIA syndromes are characterized by high brain iron with typical magnetic resonance imaging findings and the presence of axonal spheroids on histology. NBIA syndromes include some genetically still undefined conditions: PLA2G mutations were most recently added to the list of genes involved. At least 8% of PKAN patients show RBC acanthocytosis yet for other NBIA syndromes such analyses are currently not available

European Multidisciplinary Initiative on Neuroacanthocytosis (EMINA)

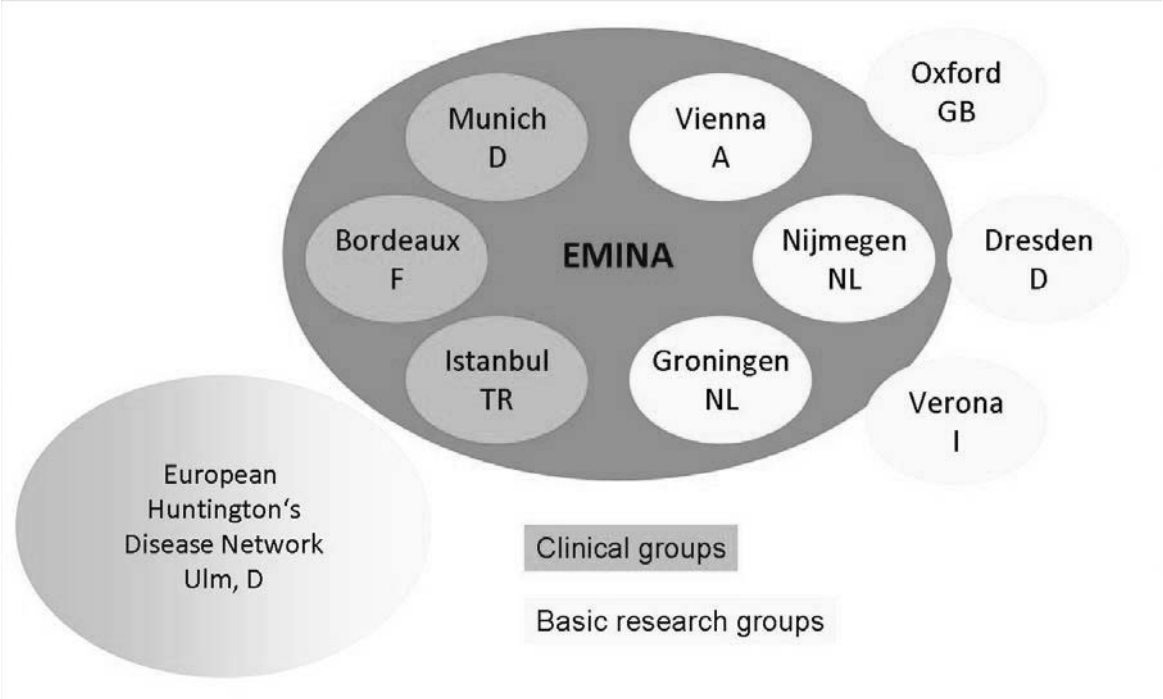
Little is known on the disease progression milestones and no treatment or cure of the debilitating disorders are available. Work with an international database should allow the development of scales for systematic treatment studies and the analysis of experience gained with procedures such as deep-brain stimulation (DBS) that is increasingly considered in the NA and NBIA syndromes.

Besides the actual mechanisms in neurodegeneration, the formation of the essentially unknown basis of the acanthocytic shape change is interesting to basic scientists. This implies alterations of membrane components of the outer and inner leaflet as compared to the disk shape of a normal RBC, the discocyte. These alterations imply a relative dilation of the outer membrane leaflet or a compression of the inner leaflet or a combination of both, outer leaflet dilatation and inner leaflet compression. Acanthocytic cells in NA blood appear to persist for

longer. Thus, one may assume the existence of stable domains within these membranes, particularly in the “thorn” regions.

One interesting aspect concerning neurons as well as erythrocytes is the role of vesiculation and autophagy in the NA and NBIA syndromes. Chorein, the VPS13A protein, appears to be involved in such processes while terminal erythropoiesis and maintenance of (neural) cell viability are both connected to autophagy. A defect in the autophagic pathway could therefore account for both neuronal and RBC dysfunction and may be studied in vitro using patient RBC membranes. Post mortem analyses of nervous tissue in the NA and NBIA syndromes on a systematic basis, using case series, are similarly needed and require appropriate collection and preservation of tissue. Nervous tissue from ChAc cases is available in the Munich brain bank and examined neuropathologically.

The development of animal models for the various disease states has become possible, yet in comparison to HD the pace has been very slow. The few existing mouse models (for MLS, ChAc, HDL2 and PKAN, lacking variety in terms of gene mutations) have not yet been exhaustively analysed and the situation appears similar for C. elegans (MLS) and Tetrahymena thermophila (ChAc). For PKAN a drosophila model has recently become available. Development of animal models for NA and NBIA syndromes clearly is a major goal in EMINA.



Partners:

- LMU - Ludwig-Maximilians-University, Neurologische Klinik und Poliklinik, Munich, Germany
- MUV - Max F. Perutz Laboratories, Medical University of Vienna, Vienna, Austria
- RUN - Radboud University Medical Center Nijmegen, Biochemistry, Nijmegen, The Netherlands

- UMCG - University Medical Center Groningen, Groningen, Cell Biology, The Netherlands
- CHUB - Centre Hospitalier Universitaire de Bordeaux, Service de Neurologie, Bordeaux, France
- ITF - Istanbul Faculty of Medicine, Department of Neurology, Istanbul, Turkey

EMINA aims and subprojects:

1. Set up NA reference centre located in Munich (Partners involved: LMU, CHUB, ITF).
2. Set up a diagnostic centre for ChAc located in Istanbul (Partners involved: LMU, ITF).
3. Analyse NA red cell membranes composition (Partners involved: MUV, RUN).
4. Analyse NA red cell proteoms and study localisation of NA relevant proteins in control cells and knock down effect on control cells (Partners involved: MUV, RUN).
5. Elucidate the mechanism of vesicle formation in erythrocytes and acanthocytes of patients with various forms of NA (Partners involved: RUN).
6. Develop Drosophila models for other NA syndromes in addition to the existing Drosophila PKAN model to identify underlying common mechanism of cell degeneration (Partners involved: UMCG).
7. Create diagnostic guidelines and taxonomy for NA syndromes including collection of NA patients (Partners involved: LMU, CHUB, ITF)

European registry and network for intoxication type metabolic diseases (E-IMD)

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Organic acidurias (OAD) and urea cycle defects (UCD) are two groups of rare intoxication type metabolic diseases (IMD) with overlapping clinical phenotype. Clinical presentation includes acute metabolic decompensation during catabolism as well as acute and/or chronic dysfunction of brain, heart, kidneys, liver, and skeletal muscle. Patients with these life-threatening diseases risk severe disability, impaired quality of life and reduced life expectancy. Because these diseases are rare, individual centres have limited experience and small numbers of patients. It is therefore necessary to pool resources for collaboration in research and public health projects and to synergise efforts.

The European registry and network for intoxication type metabolic diseases (E-IMD, EAHC no. 2010 12 01; 2011-2013) has been financed by the European DG Sanco and is a network of expert metabolic centres, sharing a registry, producing guidelines and best practice, quality assessment, training, tele-expertise and working with patient groups on evaluating and improving care. The network currently includes 78 partners from 23 countries covering more than 90% of the population in EU MS. E-IMD and UCDC (USA) have established a strategic alliance for transatlantic collaboration for patients with UCD and have planned to extend this collaboration to OAD and other IMD. E-IMD also works together with other national and international consortia such as the J-UCDC (Japan), SSIEM, patient advocacy groups, and industry.

The patient registry is one of the major tasks set up to improve evidence base and knowledge on these rare diseases (www.eimd-registry.org). It is designed in a modular way containing a core dataset for all IMD to facilitate research across disease areas. Additional individual datasets (e.g. specific biomarkers) are elaborated according to disease-specific requirements to address specific research questions for single diseases and disease groups. The registry is designed to collect longitudinal data so that the disease course of individual patients, birth cohorts and disease groups can be easily followed over time. The regular follow-up of patients includes baseline, regular annual, and emergency visits; the circumstances of fatal disease course are also reported. This visit structure is highly relevant for clinical trials, a precise description of the natural history, rare disease variants and disease modifiers, and the development of evidence-based new treatments and protocols of care, particularly in very rare diseases such as Triple H syndrome or N-acetylglutamate synthase deficiency where only 2-3 new patients may be diagnosed in Europe per year. In 2013, the IT platform will be extended to include patients with homocystinurias and methylation defects by the EU-funded project "E-HOD" (EAHC no. 2012 12 02; 2014-2016). In 2014, we plan to continue clustering of IMD by inclusion of additional diseases, then covering 49 IMD in total. Our vision is to further expand the registry and network step by step to include all IMD. There are more than 450 known different IMD in total.

E-IMD will work towards the IRDiRC objectives and follow its policy guidelines. Notably through its collaborative approach, a registry based on a philosophy of interoperability, involvement of patients in all aspects of the development and the inclusion of industry for public private partnerships.

European Research project on Mendelian Inherited Optic Neuropathies (ERMION)

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Inherited optic neuropathies (IONs) are one of the most common causes of vision loss in childhood and early adulthood affecting more than 50.000 persons in Europe with an estimated prevalence of 1:10.000. Autosomal-dominant optic atrophy (ADOA) caused by mutation in *OPA1* and Leber hereditary optic neuropathy (LHON) are the two most common IONs for which the molecular basis is currently known.
The ERMION consortium, which has been granted by the ERA-NET program E-RARE in may 2010, is composed by four teams from France, Germany and Italy.
Thanks to this grant, we have gathered the most reliable ION models (patients, fibroblasts and animal models) to allow an in-depth assessment of genetic, molecular, biochemical, histological, electrophysiological, functional and clinical parameters. These models can now be used to test the effects of therapeutic strategies, including pharmacological and gene therapies.
The main results obtained by the ERMION consortium are the following:
1. New data on natural history of IONS: 20% of patients carrying an *OPA1* mutation have extra-ocular neurological symptoms including deafness, cerebellar ataxia, peripheral neuropathy and myopathy.
2. Creation of a biobank containing more than 5000 DNA samples and more than 100 cell lines from patients with ION and creation of a public **LOVD database** (mitodyn.org) referencing OPA1 mutations (n=230) and sequence variants (n=48).
3. Identification of new ION genes and loci:
-rare primary mitochondrial DNA mutations responsible for LHON
- a new ADOA locus (OPA8)
- 4 novel genes (OPA9, OPA10, OPA11 and OPA12) responsible for non-syndromic and syndromic forms of AROA
4. Creation of an *OPA1* mouse carrying the recurrent *OPA1*(delTTAG) displaying a multi-systemic poly-degenerative phenotype
5. **New physiopathological data:** Involvement of *OPA1* in the maintenance of mitochondrial DNA and a relative resistance to neurodegereration of photosensitive retinal ganglion cells (ipRGCs).
6. **Cell lines and drug testing** on human neuronal stem cells (hNSC) deficient for OPA1,
Retinal Ganglion cell from OPA1 and OPA3 mouse models and Mouse embryonic fibroblasts (MEF) lacking OPA1 protein. More particularly, Use of resveratrol and idebenone on LHON fibroblasts showed a partial restoration of the mitochondrial complex I deficiency and use of estrogens ameliorates mitochondrial dysfunction in LHON cybrids.
7. **Clinical trials**: a retrospective study in patients affected with LHON has shown that early and prolonged treatment with idebenone significantly improves the frequency of visual recovery in the acute phase. An open-label trial of EPI-743, a novel redox modulating agent, conducted in 5 patients with LHON showed that disease progression was arrested and vision loss reversed in all but 1 of the 5 consecutively treated patients. An open-label trial of idebenone conducted during one year in 7 patients carrying *OPA1* mutation showed an improvement of color vision, shrinkage of the central scotoma and increase of visual acuity in five of seven treated patients. A double-blind controlled trial of cyclosporine in patients with LHON is ongoing.

European young investigators network for Usher syndrome: Deciphering the molecular pathogenesis and evaluate gene-based therapy strategies

Kerstin Nagel-Wolfrum* and EUR-USH network members

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Usher syndrome (USH) is a rare genetic disease and the most common form of inherited deaf-blindness in man. USH is a complex disorder divided into three clinical types, which are genetically heterogeneous. Up to now, twelve causative genes and one genetic modifier have been identified. Molecular analyses revealed that all USH1 and USH2 proteins are organized in protein networks in the eye and inner ear. Although this has provided important insights into the function of USH proteins and explains why defects in proteins of different families result in USH, the exact pathomechanisms in the retina remain unclear. Currently the auditory deficit in USH patients is successfully compensated with cochlear implants; there is no effective treatment for the retinal component of USH.

In the “European young investigators network for Usher syndrome (EUR-USH)”, young scientists with different backgrounds in medicine, genetics, cellular and molecular biology aim to synergize their expertise and bring new insights towards the understanding of USH paving the way for funded treatments and cures of USH patients.

In the EUR-USH network young European research teams will focus on a) the improvement of diagnosis, b) the deciphering of the molecular pathogenesis, and c) the evaluation of gene-based therapy strategies. For b) the USH research team in Mainz will analyze the expression profiles of USH proteins and the spatial distribution of components of their networks in the retinal photoreceptor cells of human and non-human primates. For c) the USH research team in Mainz will concentrate on the read-through therapy strategy which induces the over read of nonsense mutations by translational read-through drugs (TRIDs). We will analyze read-through of selected USH causing nonsense mutations in cell culture, organotypic retina cultures, and mice *in vivo*. Furthermore, we will assess the retinal biocompatibility of identified TRIDs and analyze application modes for targeting retinal cells in therapeutic concentration.

The aims of present EUR-USH network are to improve diagnosis, provide more insight into the molecular pathogenesis and evaluate gene-based therapy options with the ultimate goal to improve the life quality of USH patients.

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European young investigators network for Usher syndrome: state-of–the-art phenotype description, harmonized clinical documentation and Eur-USH database

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Usher syndrome (USH) is a rare disease, with an autosomal recessive pattern that results in a major handicap for the patients, since it affects the two major senses, vision and hearing. So far, nine causative genes and one genetic modifier have been identified, but ~20% USH cases remain uncharacterized. Due to the newborn deaf screening hearing deficiencies are detected early in life and the auditory deficit in USH can be compensated with hearing aids and cochlear implants. To date there is a significant USH diagnosis assessment delay, due to the differences in the ages of onset in the clinical symptoms and the rare disease character. Early diagnosis of USH supports the parents in their choice for cochlear implants instead of learning sign language. **European young investigators network for Usher syndrome (EURUSH)** is aimed to improve early diagnosis of USH, understand the molecular pathomechanism in more detail and develop treatment strategies to stop the neurosensory degeneration of the retina. A unique feature of the EURUSH is the networking between young scientists with different backgrounds in medicine, genetics, cellular and molecular biology. This is an integrating structure, which represents with an exceptionally high degree of collaborative interactions and in consequence a rapid and efficient progress of the project. Current abstract presents the combination of **state-of–the-art clinical examination protocols**, which will improve diagnosis and provide more details to genotype/phenotype correlations in this rare retinal condition. Standard USH **clinical data collection protocols and harmonization procedures** will be discussed. The large population planned to be recruited within EURUSH to describe USH disease natural history and identify progression markers based on harmonized clinical data collection. Moreover, to date existing databases and rare diseases material collections are local, small, and not accessible or standardized, which represents a crucial prerequisite for the preparation of clinical trials. Current abstract presents the **Eur-USH database** design for standardized data collection, which provides the platform for uploading the descriptive USH phenotype and genotype related data. Due to a large cohort of recruited USH patients, Eur-USH database will serve as a tool for early USH diagnosis (innovative USH screening battery). **EURUSH** will contribute for early USH diagnosis assessment, which improve the life-quality of USH patients and furthermore opens the time-window for hopefully upcoming therapeutic interventions to prevent blindness.

From Models and Mechanisms to Therapies.

Philip Hieter[1], Richard Rachubinski[2], and Paul Lasko[3], and Kym Boycott[4]

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Nature reuses the same building blocks to construct organisms as different as yeasts, worms, flies, fish, mice, and humans. Few, if any, processes at the gene level are known to be unique to humans. Indeed, key aspects of most human disorders can be modeled in experimentally tractable organisms through the analysis of orthologous genes and pathways using the genetic, biochemical and cell biological toolboxes that have been developed for each model organism.

We are at the brink of an unprecedented era of mutation discovery in humans. Next generation sequencing has significantly accelerated the pace of discovery of human genetic variation and its association with human disease.

How do we translate genomic information to advancements in diagnosis, prevention, and treatment of human disease? The answers lie in elucidating gene function and understanding how biological mechanisms are affected by specific mutations. Here is where model organism genetics, coupled with dynamic interdisciplinary dialogue and collaboration between medical scientists and basic researchers, will illuminate and be critical for decades to come.

We discuss the establishment of a Planning and Priorities Committee called "Model Organisms and Mechanisms to Therapies" supported by the Institute of Genetics of the Canadian Institutes of Health Research (CIHR). The Committee will advise CIHR, and through it, IRDiRC, on the use of model organisms to study human biology and health. The over-arching goals of the Committee are 1) to stimulate interdisciplinary dialogue between model organism researchers and clinician scientists, and 2) to enable collaborative projects, through early seed funding, that connect clinician scientists serving patients with basic scientists working with model organisms.

Title: Genetic Highthroughput Screening in Retinitis Pigmentosa Based on High Resolution Melting (HRM) Analysis.

Authors: Javier Ruiz-Ederra[1]; Olatz Barandika[1]; Ander Anasagasti[1]; Cristina Irigoyen[2]; Benitez BA[3]; Cooper B[3]; Carlos Cruchaga[3,4].

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Purpose: To test the feasibility of a high-throughput genetic screening approach for the molecular diagnosis of RP using a fast and cost-effective HRM analysis-based method, and to compare this methodology with two of the most widely used Next Generation Sequencing (NGS) platforms: Reversible dye terminator (Illumina) and Ion semiconductor (PGM) technologies.

Methods: RP patients were clinically diagnosed at the Ophthalmology Department of Donostia University Hospital, San Sebastian, Spain. We applied a genetic high-throughput screening based on HRM analysis to non-syndromic RP probands (n=115) and to a set of syndromic RP probands that met clinical diagnosis for Bardet-Biedl syndrome (BBS, n=5). A total of 17 RP genes were analyzed: 10 genes of non-syndromic RP, that in aggregate account for about 17% of all cases of RP world-wide and 7 BBS genes, that account for about 70% of all cases of BBS. Inclusion criteria for gene selection were: 1) size: genes of less than 4 kb; 2) prevalence: genes reported to account for, at least, 0.5 % of total RP cases world-wide; and 3) number of exons: genes with up to 22 exons. For comparison purposes, RHO gene was also sequenced with Illumina (GAII; Illumina) and Ion semiconductor (PGM-Ion Torrent; Life Technologies) technologies. Detected mutant variants were confirmed by Sanger sequencing and tested for co-segregation in first-degree family members of the affected proband.

Results: A total of 34 genetic variants were found, 15 of which predicted or previously reported as pathogenic. Out of these 15 variants, 5 were novel. All variants found in the RHO gene were also detected by Illumina and PGM sequencing, and were validated by direct sequencing. Furthermore, some RHO variants were detected in distant non-coding regions by HRM analysis, but not by either of the NGS platforms used, since these regions were excluded by the NGS analysis.

Conclusions: In the present work, we have validated a high throughput (HTP) genetic screening method for mutation discovery in retinitis pigmentosa (RP), based on high resolution melting (HRM) analysis. This approach has proven to be a fast and cost-effective mutation screening approach in the context of small/medium size (up to 4 kb) RP genes. All variants detected by HRM analysis were also found by two of the most widely used NGS platforms (Illumina and PGM-Ion Torrent)

Glycogen storage disease type 1a: from pathophysiology to liver gene therapy.

Coordinator: Fabienne Rajas, Inserm U855-Université Lyon 1, Lyon, France

Patients with liver-related glycogen storage diseases type 1 (GSD1) suffer from life-threatening hypoglycaemia, when left untreated. When treated by an appropriate dieting, patients' life expectancy improves considerably. Irrespective of current nutritional treatment, however, patients develop major and severe complications, such as liver cancer, end-stage renal failure and severe hyperlipidemia. Until now, the molecular mechanisms involved in these pathologies are poorly understood and available animal models do not survive after weaning. From the retrospective European study in GSD1, we learned that metabolic control appears to be essential to diminish these complications, however by mechanisms still unknown. To gain further insights into the molecular mechanisms of the disease and to evaluate potential treatment strategies, we have recently developed novel mouse models in which the catalytic subunit of glucose-6 phosphatase gene (*G6pc*) can be deleted specifically and conditionally in each glucose-producing organ. In contrast to total *G6pc* knockout mice, tissue-specific G6pc deficiency allows mice to maintain their blood glucose by induction of glucose production in the other gluconeogenic organs. Although glucose is considered mainly produced from the liver, liver G6pc$^{-/-}$ mice are perfectly viable and exhibit the same hepatic pathological features as GSD1 patients, including the late development of hepatocellular adenomas and pre-carcinomas (Mutel et al., J. Hepatol., 2011). In this project, the overall aim will be to improve the strategies of treatment on both a nutritional and pharmacological point of view and to identify new means to treat liver, kidney and intestine and metabolic complications. For that, we first propose to decipher the molecular mechanisms underlying the long-term development of the GSD1 pathology, particularly the development of hepatic, renal and intestinal dysfunctions. Since the liver is well suited for gene transfer, the second goal is to test the efficiency and harmlessness of gene therapy in the liver, using new recombinant viral vectors. Molecular studies will be performed to characterize the pathways involved in the development of hepatic tumours in liver G6pc$^{-/-}$ mice. These mice will be fed on different diets to evaluate the impact of different nutrients on the development of tumours. This will allow us studying the relationship with metabolic perturbations in the liver. The characterization of biomarkers of the processes underlying renal complications will be realized in renal G6pc$^{-/-}$ mice studied for more than 18 months. In the same way, long-term complications of intestinal G6pc$^{-/-}$ will be analysed. The liver G6pc$^{-/-}$ mouse model constitutes also a powerful tool to test the efficiency and safety of recombinant adeno-associated virus (AAV) and lentivirus. Glucose homeostasis, liver pathophysiology and hepatic tumour development will be analysed in treated mice. The first trials of liver therapy were very promising and should allow fast progress towards a new therapy of GSD1. This project will provide a better understanding of the development of hepatocellular adenomas and their possible transformation in carcinomas. The study of the liver pathology in liver G6pc$^{-/-}$ mice will permit to establish the link between specific liver metabolic deregulations in GSD1 and the development of liver tumours. This program will also enable us to provide a description of the mechanisms implicated in kidney and intestinal diseases. The outcome of these studies will be translated into new therapeutic dietary and pharmacological measures, and new promising therapeutic options, such as gene therapy, allowing us to strongly improve the long-term outcome and, thereby, the quality of life of GSD1 patients.

Hereditary chloride channelopathies of skeletal muscle and kidney: From genotype to phenotype and novel pharmacotherapeutics

Jean-François Desaphy, Antonella Liantonio, Sabata Pierno, Michela De Bellis, Paola Imbrici, Concetta Altamura, Maria Maddalena Dinardo, Giulia Maria Camerino, Roberta Carbonara, Diana Conte.

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The aims of this project include the identification of drugs for the treatment of patients suffering from chloride channelopathies, such as Myotonia Congenita (MC) affecting skeletal muscle and Bartter syndrome (BS) and salt-sensitive hypertension (SSH) affecting kidney. MC and BS are due to mutations in *CLCN1* and *CLCNKB/BSND* genes encoding chloride channels of the CLC family. Polymorphisms in *CLCNKA/KB* genes are associated with SSH. The pharmacotherapy of these diseases is purely symptomatic, while specific ClC channel ligands are dramatically lacking.
We characterized a total of 8 *CLCN1* mutations linked to recessive MC. Chloride currents were studied using patch-clamp in HEK293 cells transfected with mutant hClC-1 channels. While the pathogenic mechanism of some mutations is elucidated, it remains unclear for others. To better define the genotype/phenotype relationship, the interaction between mutants found together in compound heterozygous patients is being studied in co-transfected cell models.
The carbonic anhydrase inhibitor acetazolamide (ACTZ) is used empirically by MC patients. We tested ACTZ on hClC-1 channels in HEK293 cells. In symmetrical [Cl^-], the drug shifts the channel voltage dependence by -20 mV. Yet the drug had little effect on a hClC-1 mutant, suggesting that ACTZ may have limited effect on chloride currents in patho/physiologic conditions.
The sodium channel (NaCh) blocker mexiletine is today the first choice drug in MC, but there is a critical need for other antimyotonic drugs [Desaphy et al, *Eur J Clin Pharmacol*, 2012]. Part of our research is aimed at evaluating prompt-to-use and newly-synthesized NaCh blockers in vitro [Catalano et al, *J Med Chem* 2012; De Luca et al, *Neuromuscul Disord* 2012; Desaphy et al, *Front Pharmacol* 2012; De Bellis et al, *Biophys J*, 2013; Desaphy et al, *Mol Pharmacol,* 2013]. Using a newly-created pharmacological rat model of MC for in vivo preclinical screening [Desaphy et al, *Neuropharmacol*, 2013], we individuated a number of NaCh blockers with an antimyotonic efficacy up to 100 fold greater than mexiletine.
Regarding kidney CLC-K channels, a rational drug design allowed us to identify a newly-synthesized benzofuran derivative (SRA-36) as a very efficacious blocker of CLC-Ka currents with a 4 μM affinity constant in HEK293 cells. Surprisingly, in contrast to what observed in amphibian oocytes, niflumic acid fails to increase CLC-Ka currents in HEK cells but produces an inhibitory effect in the 1-1000 μM range. The drugs are being tested on CLC-K mutants. In parallel, in vivo studies demonstrated that acute administration of benzofuran derivatives to rats produced diuretic effects and lowered systolic blood pressure (SBP) in normotensive rats [Liantonio et al, *J Hypertens* 2012]. Preliminary results also indicated that benzofuran derivatives reduced SBP in spontaneously hypertensive rats.
Granted by telethon-Italy

Abstract IonNeurONet (IRDiRC Conference, April 2013)

Authors: Holger Lerche on behalf of the IonNeurONet consortium.

IonNeurONet, German Network of Neurological and Ophthalmological Ion Channel Disorders, is one of currently 13 research networks on rare diseases funded by the German Federal Ministry of Education and Research (Bundesministerium für Bildung und Forschung, BMBF) since 2012.

Ion channels provide the basis for the excitability of nervous and muscle tissue. Mutations in ion channel encoding genes can therefore lead to hereditary diseases with hyper- or hypoexcitability of the affected tissue. The resulting symptoms comprise muscle stiffness or weakness, migraine auras, epileptic seizures, visual defects due to retinal dysfunction, episodic ataxia, movement disorders, and episodic pain. These diseases are commonly referred to as ion channel disorders or channelopathies.

IonNeurONet partners will create a clinical and research network for these neurological and ophthalmological ion channel disorders and organise training workshops for physicians thereby improving nation-wide care for these rare and often unrecognised diseases. IonNeurONet partners will identify novel disease genes and develop a fast and efficient genetic diagnostic tool based on next generation sequencing. Additionally, partners will perform functional physiological studies in heterologous expression systems, muscle and nerve cells and provide platforms for a) genetic and bioinformatic analyses, b) automated and deep functional studies, c) channel trafficking, and d) induced pluripotent stem cells.

New Zealand's "Old MacDonald" has unique treasures down on the farm.

The New Zealand Institute for Rare Disease Research Ltd (NZIRDR) is a charitable company wholly owned by the New Zealand Organisation for Rare Disorders (NZORD).

NZORD's mission is to improve information for patients, their family and professionals, to build partnerships to improve diagnosis and clinical care, and to accelerate research towards control and cure of rare disorders. NZIRDR performs the role of promoting and supporting the research initiatives.

One such initiative is a catalogue of naturally occurring rare disease animal models, which has been established to improve interest in rare disease research and provide opportunities for such research to occur. Currently there are 29 rare disease animal models listed in NZIRDR's catalogue.

In New Zealand, there has been significant work on many of these models, leading to clinical findings that assist diagnosis and therapies for humans. Large animal models are especially good for studies involving the brain and central nervous system.

Disorder	Animal Species (Breed)
Alpha mannosidosis	Bovine (Angus and Murray Grey)
Autoimmune Lymphoproliferative Syndrome (ALPS)	Feline (British short hair)
Carwell Muscling (Muscular Hypertrophy)	Ovine (Dorset Romney cross)
Cataracts	Ovine (Coopworth)
Chondrodysplasia (Dwarfism, Spider syndrome)	Ovine (Texel)
Cystic Fibrosis	Ovine
Epidermolysis bullosa simplex (EB)	Bovine
Episodic ataxia	Ovine (Romney type)
Fecundity (Booroola)	Ovine
Fecundity (Hanna)	Ovine
Fecundity (Inverdale)	Ovine (originally Romney, but now in a number of breeds).
Fecundity (Woodlands)	Ovine
Glycogen storage disease type II (Pompe disease)	Ovine (Merino)
Hypophosphataemic rickets	Ovine (Corriedale)
Low Bone Density	Ovine (Coopworth)
Lower Motor Neuron Disease	Ovine (Open Face Romney)
Micropthalmia	Ovine (Texel)
Mucopolysaccharidosis IIIa (Sanfilippo syndrome)	Canine (Huntaway)
Mucopolysaccharidosis VI (MPSVI)	Canine (Toy Poodle)
Myophosphorylase deficiency (Glycogen storage disease type V)	Bovine (Charolais)
Neuroaxonal dystrophy	Ovine (Coopworth)
Neuronal ceroid lipofuscinosis (variant CLN5; similar to late infantile variants)	Ovine (Borderdale)
Neuronal ceroid lipofuscinosis (variant CLN6, late infantile)	Ovine (South Hampshire)
Neuronal ceroid lipofuscinosis (variant CLN6, late infantile)	Ovine (South Hampshire x Coopworth); Chimera
Polycystic kidney disease (ARPKD)	Ovine

Polycystic kidney disease (ARPKD)	Ovine (Coopworth)
Primordial dwarfism	Bovine (Angus)
Rod photoreceptor dystrophy (Day blindness)	Ovine (Wiltshire)
Segmental distal axonopathy	Ovine (Merino)

NZIRDR has plans for the discovery of more rare disease models through an active search amongst the NZ farm animal population, much of which is becoming well characterised genetically and is thus a good source for further research. NZ has one of highest companion animal ownership rates in the world and this will be investigated also.

We are interested in collaborations that might assist the rapid discovery of more large animal models for rare diseases.

Website www.nzirdr.org.nz email enquiries@nzirdr.org.nz

Phase II trial of Ang-(1-7) for the treatment of patients with metastatic sarcoma

W. Jeffrey Petty, Antonius A. Miller, and Paul D. Savage

Background: In a phase I study of angiotensin-(1-7) [Ang-(1-7)], two out of three patients with metastatic sarcoma experienced clinical benefit. This benefit was associated with reduction in plasma placental growth factor (PlGF) concentrations. Dose-limiting toxicities in the phase I study included one patient who experienced multiple small strokes. The current study prospectively examines the radiographic activity and biomarker activity of Ang-(1-7) for the treatment of metastatic sarcoma.

Methods: The study was designed to examine a continuous daily dosing schedule. Ang-(1-7) was administered by subcutaneous injection at a dose of 20 mg daily continuously until progression or unacceptable toxicities. In the event of excessive toxicities in the first cohort, a second cohort was allowed to evaluate the safety of a 10 mg daily dose. Blood samples were obtained prior to Ang-(1-7) administration on day 1, four hours after treatment on day 1, and prior to treatment on day 22. These samples were used to measure changes in PlGF, VEGF, and Ang-(1-7).

Results: The first cohort of 20 patients has completed accrual and one patient remains on study. To date, no strokes have been observed. The only dose limiting toxicity observed has been a single episode of DVT felt possibly related to Ang-(1-7). PlGF plasma concentrations for pre-treatment, 4 hour, and day 22 time points were 4.3, 4.6, and 3 pg/mL. Differences for post-treatment as compared to pre-treatment measured were not significant at the 4 hour or day 22 time points (P = .7 and P = .3). To date, no partial responses by RECIST have been observed. One patient with hemangiopericytoma

demonstrated prolonged disease stabilization (11 months). One patient with epitheliod hemangioendothelioma remains on study with a prolonged period of disease stabilization (28+ months).

Conclusions: This prospective phase II study failed to confirm the PlGF biomarker effect identified in our prior phase I study. The clinical activity manifested by prolonged disease stabilization in our cases of hemangiopericytoma and epitheliod hemangioendothelioma may warrant further investigation in these two subtypes of vascular sarcoma.

Preclinical characterization of ryanodine receptor stabilizers in muscular dystrophies

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Muscular dystrophies are characterized by primary wasting of skeletal muscle, which compromises patient mobility and can lead to complete paralysis and premature death. Our main goal is to test novel compounds that interact with calcium homeostasis on models of muscular dystrophies in vitro (on human and muring myotubes) and in vivo (on mdx and C3KO mice). Our hypothesis is that a deficit in calcium homeostasis contributes to muscular dystrophies. For that purpose, we will analyze (1) differential expression of calcium handling proteins in control and dystrophic myotube cultures and muscle sections from mice and human samples; (2) calcium homeostasis, cellular bioenergetics and citotoxicity in control and dystrophic myotubes (human and murine); (3) the effect of novel compounds on calcium homeostasis, cellular bioenergetics and cytotoxicity on dystrophic myotubes; and (4) the effect of novel compounds in vivo on mouse models of muscular dystrophy (mdx, C3KO…).
We hope that our work will help to develop new pharmacological treatment for muscular dystrophies. Moreover, we will identify some of the mechanisms implicated in muscle contractibility disorders, contributing to move forward in patient management and pharmacological treatment design.

PREVENT-ROP

New approach to preventative treatment of the blinding disease Retinopathy of prematurity (ROP)

The overall objective is to develop a novel preventative intervention for the blinding disease retinopathy of prematurity (ROP) and other complications of prematurity. This work is based on the concept that replacement of critical factor(s), normally provided in utero and reduced due to the disruption of the maternal/fetal interaction, to the infant born prematurely will help prevent the complications of premature birth.

We have shown experimentally and clinically that low IGF-I levels in premature infants strongly correlate with ROP development, indicating that IGF-I is one of these critical factors. Our preliminary work suggests that replacement of IGF-I to normal levels found in utero will prevent ROP and other complications of premature birth.

The international research project, which involves researchers from Lund University Hospital, Karolinska University Hospital, Harvard Medical School, University of Cambridge, University of Amsterdam, Institut Giannina Gaslini in Italy, the pharmaceutical company Premacure AB, the biotech company Mediagnost, and the research organisation Smerud, have now received 6 million EURO from the EU to fund further development of the project.

The project is currently performing a clinical Phase 2 study administering continous infusion of the drug Premiplex. If successful this will result in a clinical availability of a new orphan designated drug product, Premiplex®, improved care of preterm infants and a significant contribution towards the goal of the International Rare Disease Research Consortium (IRDiRC) by delivering one new therapy for the rare disease ROP.

Rapid Identification of Clinically Relevant Variants from Human Sequencing Data

R Dixon ,T Bonnert, D Richards, R. Flannery, A Kramer, A Kutchma, J Lerman, J Leschly, S Majumdar, N Marshall, M Molloy, A Muthiah, A Ning, R O'Connor, K Patel, V Rajaraman, R Rebres, A Sarver, D Bassett

Ingenuity Systems, 1700 Seaport Blvd, 3rd Floor, Redwood City, CA, 94063, US

Biological interpretation of thousands of potentially deleterious variants is a bottleneck in discovering valuable causal insights from DNA resequencing studies, often requiring months of effort after completion of the variant calling step. Ingenuity® Variant Analysis (www.ingenuity.com/variants) is an application that leverages an extensive knowledge base of millions of expert-curated mutation and biomedical findings from the literature and integrated reference information to enable real-time interactive filtering and rapid prioritization of variants. We have extended it to compute a synthesis of the current knowledge about variants to provide automated initial clinical assessments, identify variants implicated in diseases consistent with observed clinical features, and extensive coverage of pharmacogenetic impacts. Aside from empowering clinical researchers to immediately identify reportable variants in medical genomes, we have optimized gene-level burden tests on the order of 100x faster than conventional methods while delivering consistent results, and pathway level causally-consistent algorithms to find the few variants that are most compelling for follow-up in multi-sample studies. Using a combination of causal analytics, statistical and genetic analysis at the variant, gene, and pathway levels, and the ability to visualize how variants impact disease progression, we will demonstrate the application of a context-rich knowledge base to discover clinically relevant cancer driver variants and novel causal variants for human genetic disease.

Abstract IRDiRC meeting, Dublin 2013

Self-Inactivating Lentiviral Vectors for Correction of Rag1 Severe Combined Immunodeficiency

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[g]Department of Pediatrics, Erasmus MC University Medical Center Rotterdam, The Netherlands;
[h]Department of Immunology, Erasmus University Medical Center, Rotterdam, The Netherlands

Severe combined immunodeficiency (SCID) patients with an inactivating mutation in recombination activation gene 1 (RAG1) lack B- and T lymphocytes due to the inability to rearrange immunoglobulin (Ig) and T-cell receptor (TCR) genes. Gene therapy is a valid treatment option for RAG1-SCID patients, especially for patients lacking a suitable bone marrow donor, but developing such therapy has proven challenging.

As a primary preclinical model for RAG-SCID, we used *Rag1-/-* mice to test the efficacy of lentiviral SIN vectors harboring different internal elements, namely, EFS, SFFV or A2UCOE, to deliver native or codon-optimized (co) human *RAG1* or *RAG2* sequences. Treatment with select vectors resulted in the appearance of B- and T cells in peripheral blood and developing B- and T cells were detected in central lymphoid organs.

The development of B- and T cells was phenotypically confirmed in central and peripheral lymphoid organs. Serum Ig levels and TCR Vβ as well as C_μ and C_γ gene-segment usage was comparable to wild-type controls, indicating that RAG-mediated rearrangement took place. Upon stimulation of the B-cell receptor or the TCR, spleen cells showed a robust proliferative response and cytokine production. In addition, i*n vivo* challenge with TNP-KLH resulted in production of TNP-specific antibodies, confirming correct cooperation of B and T cells. Toxicity related to ectopic RAG1 expression was not observed.

We compared the native and codon-optimized *RAG1*-vectors *in vivo.* For the SFFV-driven vectors we found, on a per-copy basis, an 18-fold higher transgene expression in animals that received cells transduced with the SFFV.coRAG1 when compared to the same backbone carrying the native RAG1 sequence. Moreover, animals treated with the codon-optimized RAG1 gene showed more robust T and B cell reconstitution. Therefore we conclude that codon optimization might be of critical importance to reach sufficient *RAG1* expression, while refraining from high VCNs.

Next, we compared the capacity of the A2UCOE.coRAG1 vector to the SFFV.coRAG1 vector. Evaluating transgene expression per vector copy in BM, thymus and spleen cells, 20 weeks after transplantation, the UCOE.coRAG1 gave a 5 to 20-fold higher expression per integrated vector than SFFV.coRAG1. These properties allowed for correction of the *Rag1-/-* phenotype, while limiting the VCN. In that respect, the use of the UCOE.coRAG1 SIN lentiviral vector looks promising for clinical application.

The qualitative regeneration of the B- and T-cell compartment provides proof-of-principle for therapeutic RAG1 gene-transfer in Rag-deficient mice using lentiviral SIN vectors. Combining the choice of promoters or promoter-like elements with the codon-optimization of the *RAG1* coding sequence has brought lentivirus-based gene therapy for RAG-SCID patients closer to clinical application. Our current efforts are aimed at lentiviral RAG1 gene transfer into human CD34+ hematopoietic stem/progenitor cells and to see correction of the phenotype in CD34+ cells from RAG1-SCID patients transplanted into the murine NSG xenograft model.

Spanish Rare Diseases Registries Research Network (Spain-RDR)

Posada de la Paz M, Villaverde-Hueso A, Alonso V, Hens M, Morales A, Zurriaga O, Astray J, Aldana-Espinal JM, Margolles MJ, Jiménez J, Palomar JA, Santana M, Ramalle-Gomarra E, Ramos JM, Arribas FE, Álamo R, Gutiérrez-Ávila G, Galmés A, García Ribes M, Navarro M, Izarzuaga MI, Ardanaz ME y Abaitua I

The *Spanish Rare Diseases Registries Research Network*-SpainRDR is a project financed by the Institute of Health Carlos III (ISCIII) for the years 2012 to 2014. ISCIII is a full member of IRDiRC. SpainRDR aims to build the National Rare Diseases Registry in Spain based on the input of two different strategies: patient registries addressed to patient outcome research and population-based registries addressed to epidemiologic research and social and health systems planning. This project involves all Health Departments of the Autonomous Communities (regions) of Spain, the Spanish Ministry of Health, the Spanish Centre of Reference for People and Families affected by Rare Diseases (CREER), Spanish Medical Societies, four research networks, pharmaceutical and biotechnological organizations (ASEBIO, AELMHU and FARMAINDUSTRIA), the Spanish Federation of Rare Diseases (FEDER) and its foundation (FEDER TELETHON). The Institute of Rare Diseases Research (IIER) acts as coordinator and leader of this network. The project is organised in six work packages: WP1 Coordination and Management, WP2 Registering activity related methods, WP3 Data analysis and outcomes research, WP4 Quality Assessment and ethical and legal issues, WP5 Dissemination and impact, and WP6 Patient registries. The overall gain of the Spanish National Rare Diseases Registry will provide the necessary information to contribute to improve prevention, diagnosis, prognosis and new treatments as well as a better quality of life of patients and their families.

Support to the International Rare Diseases Research Consortium : a new service to the research commmunity

S. Aymé, B. Cagniard, R. Favresse, S.Peixoto, N. Lévy

The International Rare Diseases Research Consortium (IRDiRC) joins members that share common goals and principles and have agreed to work in a coordinated and collaborative manner within a multinational consortium. The objective is to team up researchers and organisations investing in rare diseases research in order to achieve two main objectives by the year 2020, namely to deliver 200 new therapies for rare diseases and means to diagnose most rare diseases. The members are research funding organizations dedicating over 10 million US$ to research into rare diseases. A number of challenges will need to be addressed through collaborative actions: establishing and providing access to harmonised data and samples, performing the molecular and clinical characterisation of rare diseases, boosting translational, preclinical and clinical research, and streamlining ethical and regulatory procedures. The consortium has established three Scientific Committees. The Diagnostics committee advises on research related to the diagnoses of rare disease, including sequencing and characterization of these diseases. The Interdisciplinary committee provides expertise on cross-cutting aspects of rare diseases research including issues related to ontologies, natural history, biobanking, and registries. The therapies committee gives guidance for the pre-clinical and clinical research aiming to deliver new therapies for rare diseases. The guiding principles, the plan for action and the achievements so far will be presented, as well as the way for the genetic research community to get engaged in this global effort. The scientific secretariat of this International consortium is established at the rare Disease Platform in Paris.

TREATMENT OF ADRENAL INSUFFICIENCY IN NEONATES AND INFANTS

TAIN stands for **T**reatment of **A**drenal **I**nsufficiency in **N**eonates and infants and is supported by the European Commission through its 7th Framework Programme

The aim of TAIN is to develop a new formulation of hydrocortisone – Infacort® - that can be used from birth and specifically in the age range 0 – 2 years (neonates & infants). Hydrocortisone is an essential glucocorticoid hormone used as a replacement therapy for the treatment of the rare disease Adrenal Insufficiency - where the body cannot produce sufficient cortisol. The problem of effective hydrocortisone replacement is especially acute in this young patient range where no licensed therapy exists.

TAIN involves European leaders in drug development, neonatology and paediatric pharmacology.
A Paediatric Investigation Plan has been developed that will enable clinical trials to be carried out to provide sufficient evidence of safety and efficacy for Infacort® to allow submission of a Paediatric Use Medicines Authorisation to the European Medicines Agency.

TAIN also aims to raise awareness of Adrenal Insufficiency by working closely with parents, patient groups and clinicians. This will allow the work being conducted through TAIN to benefit the widest possible amount of children, their families and health professionals.

Scientific coordinator:
Martin Whitaker, University of Sheffield, United Kingdom (martin.whitaker@sheffield.ac.uk)

Participating countries: UK (Coordinator), CH, DE

Project website: http://www.tain-project.org/

Duration: from December 2011 to November 2015

Total project cost: €5.6 million

Total EU contribution: €4.2 million

Project Number: 281654 (FP7-HEALTH)

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THALAMOSS: THALAssaemia MOdular Stratification System for personalized therapy of beta-thalassemia – from OMICS to personalized treatment of thalassemia.

Roberto Gambari – Department of Life Sciences and Biotechnology, Ferrara University, Italy

INTRODUCTION. The β-thalassaemias are a group of severe and rare anaemias with monogenic inheritance, a complex systemic phenotype and treatment-related complications. Novel and mostly experimental treatments, such as the chemical induction of the endogenous β-like γ-globin gene or the restoration of β-globin levels by gene therapy, show great promise but significant variability of success between patients. A small number of modifiers with significant impact on disease penetrance, severity and efficacy of treatments are known, but most remain elusive. Improvements of existing treatment regimens and optimisation and application of novel treatments will critically depend on the characterisation of additonal disease modifiers and the stratification of patients for customised treatment regimens.

AIMS. THALAMOSS (www.thalamoss.eu, started 11/2012) aims develop a universal sets of markers and techniques for stratification of β-thalassaemia patients into treatment subgroups for (a) onset and frequency of blood transfusions, (b) choice of iron chelation, (c) induction of fetal hemoglobin, (d) prospective efficacy of gene-therapy.

WORKPACKAGES. THALAMOSS is organized in the following Workpackages: WP1. Recruitment, patient characterization and development of culture technologies for erythroid precursor cells [WP Leader: UNIFE]; WP2. Omics analyses [WP Leader EMC]; WP3. Novel therapeutic approaches [WP Leader: CING]; WP4. Data management and analysis [WP Leader: MU]; WP5. Dissemination and exploitation [WP Leader: BRFAA]; WP6. Regulatory and ethical issues [WP Leader: BIOCEP]; WP7. Program management [WP Leader: UNIFE].

IMPACT. The impact of THALAMOSS is the provision of novel biomarkers for distinct treatment subgroups in β-thalassaemia (500–1000 samples from participating medical centres), identified by combined genomics, proteomics, transcriptomics and tissue culture assays, the development of new or improved products for the cell isolation, characterisation and treatment of β-thalassaemia patients and the establishment of routine techniques for detection of these markers and stratification of patients into treatment groups.

EXPECTED PRODUCTS. Translation of these activities into the product portfolio and R&D methodology of participating SMEs will be a major boost for them as well as for the field. THALAMOSS tools and technologies will (a) facilitate identification of novel diagnostic tests, drugs and treatments specific to patient subgroups and (b) guide conventional and novel therapeutic approaches for β-thalassaemia, including personalised medical treatments.

KEY RESEARCHERS of THALAMOSS are R.Gambari (Ferrara University, Italy, UNIFE), M. Kleanthous (The Cyprus Foundation for Muscular Dystrophy Research, Cyprus, CING), S.Philipsen (Erasmus Universitair Medisch Centrum Rotterdam, The Netherlands, EMC), E.Katsantoni (Biomedical Research Foundation, Academy of Athens, Greece, BRFF), S.Rivella (Weill Cornell Medical College, NY, USA, CU), P.Holub (Masaryk University, Czech Republic, MU), R.Galanello (Cagliari University, Italy, UNICA), SL.Thein (King's College Hospital, UK, KCL), E.Voskaridou (Laiko General Hospital, Greece, LGHA). Participating SMEs are Biocep (Israel), NovaMechanics Ltd. (Cyprus) and IRBM (Italy). Industrial activities are also provided by Harbour Antibodies (HA, The Netherlands). ThalaMoSS is financed through the FP7-HEALTH-2012-INNOVATION-1 call, project number 306201. **WEB site.** www.thalamoss.eu.

MEETINGS. The first THALAMOSS Scientific Meeting (*From Basic Research to Novel Applications on the Road to Personalized Treatment of Thalassaemia*) was organized in Ferrara, January 14, 2013.

THALAMOSS publications: (1) Macrophages support pathological erythropoiesis in polycythemia vera and β-thalassemia. Ramos P, Casu C, Gardenghi S, Breda L, Crielaard BJ, Guy E, Marongiu MF, Gupta R, Levine RL, Abdel-Wahab O, Ebert BL, Van Rooijen N, Ghaffari S, Grady RW, Giardina PJ, Rivella S. Nat Med. 2013 Mar 17. doi: 10.1038/nm.3126. (2) Induction of erythroid differentiation and increased globin mRNA production with furocoumarins and their photoproducts. Salvador A, Brognara E, Vedaldi D, Castagliuolo I, Brun P, Zuccato C, Lampronti I, Gambari R. J Photochem Photobiol B. 2013 Feb 27;121C:57-66. doi: 10.1016/j.jphotobiol.2013.02.011.

The Canadian Inherited Metabolic Diseases Research Network: A Pan-Canadian, Practice-Based Research Network for Inborn Errors of Metabolism

Beth K Potter[1], Pranesh Chakraborty[2], Doug Coyle[1], Jonathan B Kronick[3], Kumanan Wilson[4], Cheryl Greenberg[5], Monica Hernandez[6], Sara Khangura[1], Anne-Marie Laberge[7], Julian Little[1], Jennifer MacKenzie[8], Aizeddin Mhanni[5], John J Mitchell[9], Chitra Prasad[10], Kathy N Speechley[10] Sylvia Stockler[11], on behalf of the Canadian Inherited Metabolic Diseases Research Network

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Inborn errors of metabolism (IEM) are a group of >400 rare genetic metabolic diseases. Similar to other rare diseases, challenges to providing evidence-informed care for IEM include: a limited understanding of their natural history; few resources for developing and accessing interventions; and difficulties in conducting robust research. To address these challenges, we have established a pan-Canadian research network to develop new approaches to data collection and analysis that will support evidence-based care for pediatric patients and their families.

The Canadian Inherited Metabolic Diseases Research Network (CIMDRN) is a multidisciplinary group of approximately 40 investigators whose expertise spans the fields of metabolic medicine, clinical evaluative science, and health economics and policy, and whose membership represents all major pediatric metabolic treatment centres in Canada. We are guided by a novel practice-based research framework that emphasizes rigorous assembly and analysis of observational data to document variation in care and outcomes for children with a broad range of approximately 30 IEM, including diseases incorporated into expanded newborn screening and those identified as priorities by both Canadian and international research groups. To support this work, we are developing a collaborative, consent-based research platform that gathers longitudinal data from a variety of sources, including provincial policy information, health system administrative and clinical data, and self-reported patient and family experiences. The research platform is founded, in part, on preliminary work that included a survey to characterize the system of care for pediatric IEM in Canada, which presents unique challenges and opportunities due to our large and geographically dispersed population (~34 million people).

CIMDRN has identified three common themes capturing priority research questions for IEM: (1) tailoring care in the context of clinical heterogeneity; (2) a shift in priority from prevention of severe negative outcomes to achievement of optimal outcomes; and (3) the need to critically evaluate the comparative effectiveness of emerging and established therapies. Our research framework integrates these themes with a range of outcomes to encompass clinical, patient/family, and health system perspectives. Using our research platform, we are developing a comprehensive population-based description of the epidemiology and distribution of health care interventions and outcomes for Canadian children with IEM. We are also investigating associations between specific patterns of interventions and outcomes, incorporating natural history modeling approaches and developing case studies based on our priority research themes.

CIMDRN's research will lead to improved care and outcomes for patients with IEM while being mindful of the impacts on the health care system; and will generate unique methodological insights that can be applied to clinical and health policy evaluative research in other rare disease settings in Canada and internationally.

The European Genome-phenome Archive

Justin Paschall, Ilkka Lappalainen, Vasudev Kumanduri, Jeff Almeida-King, Paul Flicek

European Bioinformatics Institute

The European Genone-phenome Archive (EGA) is the primary archive at the EBI for data from biomedical research projects for which study participants have signed informed consent. EGA allows data to be distributed through a secure process to researchers in accordance with an approved data access agreement. The EGA works with Data Access Committees to distribute data for authorized users and provides interactive and secure tools for data submissions. Phenotype data can be associated with the samples. As part of an international collaboration, the EGA works together with dbGaP from NCBI to provide the most comprehensive catalogue of control access studies in the world. As of September 2012 data from more than 230 studies and 200 000 samples have been stored in the EGA. Studies include array-based genotype and raw reads from exome/full genome re-sequencing and transcriptome experiments. Within the context of RD-Connect , the EBI will archive data generated by the project in the EGA database and extend the prior collaboration among the partners to streamline and facilitate the banking of the shortreads of the B-Projects and the other IRDiRC projects.

Farmer A, Ayme S, Maffei P, McCafferty S, Sinnott R , Mlynarski W, Nunes V, Paquis V, Parkinson K, Tillmann V, Barrett T.

The Euro-WABB Registry www.euro-wabb.org : a rare disease registry for Wolfram, Alström, Bardet-Biedl and other rare diabetes syndromes.

Objectives. We aimed to develop a registry for the rare genetic diseases Wolfram (WS), Alstrom (AS), Bardet Biedl (BBS) and other diabetes syndromes, containing clinical, genetic diagnostic and outcome data. The purpose is to establish the natural history of these diseases; to assess clinical management; to characterize cohorts for future clinical trials; and to establish genotype phenotype relations. This abstract describes the first 115 patients recruited. Methods. Patients with a confirmed diagnosis (clinical or genetic) were recruited from both within and beyond Europe by their physicians. Information was collected for 42 'core' data fields, reached by consensus to differentiate between syndromes. We analysed prevalence of core clinical symptoms including obesity and diabetes. Results. The age range was 2-44 yrs (interquartile range 10-22yrs). There were 52 patients with WS (median age 19yrs (range 4-45yrs)), 29 with AS (17 yrs (3-54yrs)), 31 with BBS (10yrs (2-20)), 2 with Wolcott-Rollison and 1 with vision and hearing impairment of unknown cause. Mutations in WFS1 and ALMS1 genes were identified in >95% of patients with WS and AS, but in BBS only 70% of patients had mutations in known BBS genes. The prevalences of obesity and median ages of onset were: WS (1/52; 8yrs); AS (21/29; 1); BBS (25/31; 1yrs); $p< 0.001$ for obesity prevalence WS vs AS and BBS combined). The prevalences of diabetes and median ages of onset were: WS (46/52; 5yrs); AS (9/29; 15yrs); BBS (2/31; 12 yrs); $p< 0.01$ for ages of onset WS vs AS and BBS combined). Conclusions. The core dataset captured sufficient data to differentiate between diabetes syndromes. Diabetes mellitus presented before puberty in WS, was not associated with obesity, and is known to be insulin dependent; whereas it presented during puberty in AS and BBS, was associated with obesity, and is insulin resistant. The prevalence of diabetes is low in AS and BBS during childhood. Further patient recruitment and longitudinal data collection will use a consensus extended dataset of 400 fields to accurately characterize the phenotypes.

IRDiRC 2013 Conference

ABSTRACT Submission

The Genetic and Rare Diseases Information Center (GARD): Eleven Years of Improving Access to Hard-to-Find Genetic and/or Rare Diseases Information and Resources

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The Genetic and Rare Diseases Information Center (GARD) provides the public with access to current, reliable, and often hard-to-find information about genetic and/or rare diseases in English or Spanish. GARD is funded by two components of the National Institutes of Health (NIH), the Office of Rare Diseases Research, which is part of the National Center for Advancing Translational Sciences (ORDR-NCATS), and the National Human Genome Research Institute (NHGRI). Over the last 11 years, GARD has responded to more than 38,000 requests for information. The public can call or write to request information about rare diseases - diseases that affect fewer than 200,000 people in the United States - or about conditions that may be genetic but not rare. Questions are answered by GARD's experienced Information Specialists that include genetic counselors and a medical geneticist. Experts in genetic and/or rare diseases from NIH and a cytogenetic expert are also available to GARD staff as technical advisors.

Many people coping with a rare disease must become an expert on their disease due to limited access to or knowledge about health care providers with expertise in the condition. Although health information is increasingly available via the internet, information about genetic and/or rare diseases is still hard to find or very technical. GARD serves as a useful source of information for people who need help understanding information they receive from their health care provider or learn from their own research. Patients and their family members or friends make up two-thirds of the people who contact GARD. Another 11% are health care providers. People often request help finding genetic tests, treatment information, ongoing research efforts, and experts in a particular condition. GARD provides each user with a customized response including answers to questions in plain language, referrals to NIH and other federal resources and publications, high quality Web sites, advocacy organizations, experts, clinical trials, and genetic services.

In addition to providing timely access to Information Specialists via multiple channels, GARD also provides online access to its extensive database (http://rarediseases.info.nih.gov/GARD/). Each of the more than 6,300 diseases on the ORDR-NCATS rare disease list has its own Web page where GARD Information Specialists post information, resources, and answers to questions that GARD receives. These Web pages include an expanding variety of resources including management

guidelines, CLIA-certified genetic testing laboratories, approved orphan products, medical literature searches, disease nomenclature review and more. GARD's experienced Information Specialists and growing online collection of resources and Q&As are useful tools for patients and health care providers to quickly find the resources and services they need to help them to manage genetic and/or rare diseases.

TRANSPOSMART : an innovating platform using transposon and S/MAR for von Willebrand disease gene therapy

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Type 3 von Willebrand disease, which has a prevalence below five per million, is caused by the absence of the haemostatic protein von Willebrand factor, leading to a severe bleeding disorder. Since the disease treatment requires high blood levels of von Willebrand factor, a therapeutic approach ensuring highly efficient and prolonged in vivo production of the missing protein would be of particular benefit for the patients in terms of health, comfort of life and cost.

Liver is an optimal organ for the secretion and systemic distribution of a therapeutic transgene product and has been shown to efficiently express the functional transgene von Willebrand factor. The TranspoSMART consortium will assemble and fine-tune “cutting edge” gene therapy tools in combination with either a non-integrating approach or a transposon-based integrating approach for high and sustained level of therapeutic protein secretion by the liver. Each partner is an expert in one of the key technologies to be integrated: pFAR biosafe miniplasmids, hyperactive Sleeping Beauty transposon system SB100X, adenovirus/ transposon hybrid technology, Scaffold/Matrix-Attachment Region, and gene delivery to the liver by either plasmid hydrodynamic or adenoviral techniques. Our consortium gathers recognized leaders in the field of gene therapy and of von Willebrand disease pathophysiology. The consortium will apply the added value of this integrated platform to mouse and dog models of von Willebrand disease.

Our approach offers an optimal strategy to deliver and express the von Willebrand factor-encoding gene in the liver, for blood secretion. The dog study represents a promising translational step towards a clinical trial for the gene therapy of von Willebrand disease and ultimately for a number of gene therapy applications.

TREAT-CMT

Traslational Research, Experimental Medicine and Therapeutics of Charcot-Marie-Tooth Disease

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TREAT-CMT is the Spanish Consortium on Charcot-Marie-Tooth disease (CMT) set up in March 2012. It is based on **multidisciplinary integrating cooperation of twelve clinical and basic research groups** working with the aim of improving the diagnosis and treatment and thus of the clinical approach and quality of life of people affected by CMT. TREAT-CMT is coordinated by the Centre for Biomedical Network Research on Rare Diseases (CIBERER) and financed by the Instituto de Salud Carlos III (ISCIII) in the framework of the International Rare Disease Research Consortium (IRDiRC). The purpose and activities of this coordinated group focus on **three clinical and scientific objectives:**

1. **Research into natural history, clinical phenotyping and generation of clinical tools.**
   - *Establishment of common criteria and procedures for research on natural history and the phenotype of patients with specific clinical forms of CMT.*
   - *Neuropathological phenotyping.*
   - *Integration of clinical databases and generation of a Spanish database of CMT mutations.*
   - *Setting up a distributed biobank of biological samples.*

2. **Translational genetics and genomic studies for discovering new CMT genes and identifying biomarkers.**
   - *Genomic mapping and exome sequencing.*
     - Characterising new genes and new mutations involved in CMT and developing tools for exhaustive global diagnosis.
     - Identifying genetic modifiers in patients and their families with mutations in the GDAP1 gene and carriers of the CMT1A duplication.
   - *Biomarkers in CMT neuropathy*
     - Identifying marker proteins of CMT neuropathy connected with oxidative stress & energy metabolism.
     - Identifying metabolites acting as biomarkers in CMT neuropathy.

3. **Physiopathology and therapeutics of CMT forms associated with mitochondria.**
   - *Generation and characterisation of models of mitochondrial CMT disease models.*
     - Human and mouse cell culture models (Gdap1^{loxP} knock-out and Mfn2^{R94Q} transgenic mice)
     - Mouse (Conditional KO Gdap1loxP, Transgenic mouse Mfn2R94Q)
     - Drosophila
   - *Calcium metabolism and preclinical tests in mitochondrial CMT.*

The consortium also focusses its work on social and ethical aspects with a major vocation for diffusion of its activities and participation of patients' organisations. Coordination is another essential element and will take place in the framework of the CIBERER as a centre agglutinating cooperative research.

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3D RD: 3D facial analysis for Rare Diseases

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Fine scale, objective, scalable, portable, cost efficient, and preferably non-invasive and non-irradiating phenotypic assessments are required to complement –omics investigations; including for exploration of genotype-phenotype relationships. Optical scanning configured to capture 3-dimensional form, and coupled with engineered geometric morphometric tools (3D facial analysis) fulfills these criteria and is particularly suitable to investigations of rare diseases (RD).

The face is a biological billboard that reflects systemic health and provides diagnostic clues to individual disorders. Multiple arguments support the application of 3D facial analysis to the study of rare diseases:

- Some rare conditions have diagnostic facies that have been objectively documented (1, 2).
- Novel approaches to 3D facial analysis have been developed that approach the issue of the rarity of individual RD (3, 4). These techniques allow for a more individualized assessment of facial dysmorphology. One technique, Dysmorphometrics, uses Expectation-Maximisation modelling when projecting individual or cohort data onto a shape-space of reference data to enable the definition of a facial anomaly in an objective, quantifiable and reproducible manner. It has been used to establish a facial signature for a rare and treatable disorder that had variable severity proportional to disease state. This finding potentially provides adjunctive evidence for non-invasively monitoring treatment response (2). Further support for this concept is documentation of progressive reduction in facial dysmorphology with treatment (in prep). With the expansion of RD therapeutics, developments in treatment monitoring are required. Equally for those disorders for which treatments are not currently available, these techniques will be important for objective natural history studies(5).
- It can be employed to objectively plan facial surgery and monitor its outcomes(6).
- Joint investigations with traditional imaging technologies can be performed. (7).
- It has been used to investigate disease biology (8).
- Methods have been developed to enhance assessment of key components of facial development including symmetry and asymmetry (Figure 4) (9). Notably, facial asymmetry is commonly associated with rare dysmorphic conditions and when compared to the normative range, there are differential patterns of asymmetry in rare syndromic disease (in prep).

- It has been suggested as a means to investigate monogenetic and epigenetic disorders(10), as well as complex phenotypes including vaccine responses(11).

The Cranio-Maxillo-Facial Unit at Princess Margaret Hospital for Children and Genetic Services of Western Australia, together with colleagues from the Catholic University Leuven, Belgium, have collaborated to develop tools utilising 3D facial data ascertained from an extant and growing (n=approx 1250) reference set of individuals, without disorders known to affect the face, to delineate and investigate normal variation. Analyses of this normative reference has provided the foundation to investigate disorder specific data sets in addition to an unselected, for specific disorders, syndromic and rare diseases dataset.

In conclusion, 3D facial analysis provides a facial phenomic assessment that is uniquely applicable to studies of rare diseases, including for combination with other –omics technologies.

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PhenoTips: patient phenotyping software for clinical and research use

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We have developed PhenoTips: open source software for collecting and analyzing phenotypic information for patients with genetic disorders. Our software combines an easy-to-use interface, compatible with any device that runs a Web browser, with a standardized database back-end. The PhenoTips' user interface closely mirrors clinician workflows so as to facilitate the recording of observations made during the patient encounter. Collected data include demographics, medical history, family history, physical and laboratory measurements, physical findings, and additional notes. Phenotypic information is represented using the Human Phenotype Ontology; however the complexity of the ontology is hidden behind a user interface which combines simple selection of common phenotypes with error-tolerant, predictive search of the entire ontology. PhenoTips supports accurate diagnosis by analyzing the entered data, then suggesting additional clinical investigations and providing OMIM links to likely disorders. By collecting, classifying and analyzing phenotypic information during the patient encounter, PhenoTips allows for streamlining of clinic workflow, efficient data entry, improved diagnosis, standardization of collected patient phenotypes, and sharing of anonymized patient phenotype data for the study of rare disorders. Our source code and a demo version of PhenoTips are available at http://phenotips.org.