Molecular Syndromology

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INTERNATIONAL CONGRESS ON RESEARCH ON RARE AND ORPHAN DISEASES

29th February – 2nd March
Gehry Building, Norvartis Campus
Basel, Switzerland

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Molecular Syndromology

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Introduction

In the future, everyone will be world famous for 15 minutes.
A. Warhol

Who Said Orphan Diseases?

Answering this question could possibly turn into a 'black swan event': an unpredictable, rare event, carrying an extreme impact and playing an important role for the future. Increasing public awareness and financially supporting the research on rare and orphan diseases are the main goals of the BLACKSWAN Foundation (www.blackswanfoundation.ch). Unfortunately, there is a lack of funding of research programs in this field. My first idea as president of the BLACKSWAN Foundation was to generate a unique event, which will bring together courageous researchers working on rare and orphan diseases, thus demonstrating to the large public that many researchers based all around the world are now dedicating their time and efforts to better understand a particular rare disease.

In Switzerland, the Gebert Rüf Stiftung, which is active in various innovative fields, runs the initiative 'Rare Diseases – New Approaches' based on annual calls with a budget of CHF 2 million per year since 2009. The program aims at developing and implementing innovative technologies or approaches in the diagnosis and treatment of rare diseases (www.grstiftung.ch). The collaboration between our two Foundations to organize the first international congress on research of rare and orphan diseases was an obvious breakthrough!

February 29th was chosen to mark Rare Disease Day by EURORDIS, the European Organization for Rare Diseases. This year, this rare day will again serve to raise awareness of the plight of patients with rare disorders.

Rare diseases fail to attract commercial research efforts because of the small number of people affected. In Europe, a disease or disorder is defined as rare when it affects less than 1 in 2,000 individuals. There are between 6,000 and 8,000 rare diseases. On the whole, rare diseases may affect 500 million individuals around the world. Eighty percent of rare diseases are of genetic origin and are often chronic and life-threatening. As a result, only a handful of scientists, often working in isolation, focus their research on a particular rare disease. The dedication of scientists to a rare disease can deliver groundbreaking insights into more general disease processes. Exposure of molecular mechanisms underlying rare diseases, therefore, has the potential to help many other patients.

The RE(ACT) Congress 2012, the first international congress dedicated to rare and orphan diseases, was held in Basel, Switzerland (February 29–March 2, 2012). Its venue, the Gehry Building on the Novartis Campus, is a superb setting to stimulate learning, exchange and networking. The congress has brought together world leaders and young scientists from the academic and industrial worlds. They work on topics as diverse as stem cells, cell biology, gene therapy, human genetics, and therapeutic applications and presented cutting edge research, discussed recent results and exchanged ideas. The congress goals are to promote research on rare and orphan diseases among the general public, industry and policy makers, and to bring together researchers and their knowledge to help support the understanding of other more common diseases and encourage clear insights.

Let us all together take this opportunity to act, to RE(ACT). We are happy to share the abstracts with you that were presented during the 3-day RE(ACT) Congress by almost 300 participants from all around the world. Please do enjoy them, and with Andy Warhol in mind, maybe everyone will be world famous for 15 minutes!

'Rendez-vous' for the next edition in 2014!

For more information and to watch a selection of the presentations by the keynote speakers visit our website: www.react-congress.org.

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Gene and Cell Therapy; Stem Cells

A001_2012

Effect of Tetrahydrobiopterin and Pharmacological Chaperones on Tyrosine Hydroxylase: Correction of Neurotransmitter Deficiencies

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Tyrosine hydroxylase (TH) is the rate-limiting enzyme in the synthesis of dopamine, noradrenaline and adrenaline. Primary inherited defects in TH have been associated with L-DOPA responsive dystonia (DRD) and infantile parkinsonism. We have shown that both, tetrahydrobiopterin (BH4) and a pharmacological chaperone (compound III; a small compound that rescues misfolded proteins), stabilize in vitro human TH as well as mutants associated with DRD. Supplementation of mice with either BH4 or compound III increases total TH activity and protein in mouse brain. This increase was not accompanied by changes of steady-state brain levels of dopamine and monoamine neurotransmitter metabolites DOPAC and HVA. The failure to observe an increase in dopamine, despite a higher total TH activity, might reflect the strict enzyme regulation in vivo. Nevertheless, we anticipated that increased TH activity will lead to increased dopamine synthesis in certain conditions, including pathological states. We have investigated this possibility using mouse models of phenylketonuria (PKU) where synthesis of neurotransmitters is compromised due to high concentration of L-Phe in brain ([Phe] is 5-fold higher in ENU1/2 than in normal C57BL/6 mice). Supplementation with either BH4 (50 mg/kg/day) or compound III (8 mg/kg/day) for 10 days increases TH activity and protein in brain of ENU1/2 mice on normal diet, and we also found a trend towards elevated content of dopamine in mice treated with compound III. These treatments might be promising as therapy for disorders associated with TH misfolding and other deficiencies in dopaminergic neurotransmission.

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A002_2012

Restoration of Anti-*Aspergillus* Defense by NETs in Human CGD after Gene Therapy Is Calprotectin-Dependent

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Introduction: Infection by *Aspergillus* spp. is a potentially lethal disease in patients with neutropenia or impaired neutrophil function. We showed previously that *Aspergillus nidulans* hyphae which are too large for neutrophil phagocytosis are growth inhibited by reactive oxygen species-dependent formation of neutrophil extracellular traps (NETs). NETs are composed of chromatin (DNA and histones) and intracellular antimicrobial substances, liberated by activated neutrophils for trapping of microbes and concentrated antimicrobial defense. This process is defective in a genetic phagocyte defect, chronic granulomatous disease (CGD), due to impaired phagocyte nicotinamide adenine dinucleotide phosphate (NADPH) oxidase function. The antifungal agent responsible for *A. nidulans* growth inhibition within NETs has not been characterized. **Methods:** Antifungal activity of free and NET-released calprotectin (S100A8/A9) was assessed by incubation of *A. nidulans* with purified calprotectin, induced NETs from control and FACS (gp91phox) sorted CGD neutrophils after gene therapy (GT) in presence or absence of Zn^{2+} or α-S100A9 antibody, and with induced NETs from wild type or S100A9$^{-/-}$ mouse neutrophils. **Results:** We identified the host zinc-chelator calprotectin as neutrophil-associated antifungal agent expressed within newly formed NETs after reconstitution of NADPH oxidase function by GT for human CGD. Calprotectin prevents *A. nidulans* growth reversibly at low concentration, and leads to irreversible fungal starvation at higher concentration. Reconstituted NET-formation was associated with rapid cure of pre-existing therapy refractory invasive pulmonary aspergillosis in vivo. **Conclusion:** These results demonstrate the critical role of NET-associated calprotectin in human innate immune defense to combat invasive *Aspergillus* infection.

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A003_2012

Study of the *ACVR1* Gene Expression and Regulation

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ACVR1 encodes a BMP type I receptor mutated in fibrodysplasia ossificans progressiva, a rare and severe autosomal dominant form of heterotopic ossification. Elements such as the promoting region as well as transcriptional and post-transcriptional mechanisms regulating *ACVR1* expression are still unknown and are the object of our studies. According to data available in GenBank, *ACVR1* has 2 main transcripts differing in their 5′ UTR end. Our bioinformatic analysis of the genomic region containing the gene reveals the presence of several ESTs, predicting the existence of multiple transcripts in which different 5′ UTR exons are combined to a common coding sequence. The 3′ UTR region is common to all transcripts and contains AU-rich elements and putative, well-conserved binding sites for miRNAs. Following the above prediction, we found transcripts with different exon composition at the 5′ UTR and show their expression profile in different tissues. These data suggest complex regulation, with different transcription start sites (TSS) and promoter regions and possible elements controlling transcript stability or translation. Our work has allowed the identification of a TSS common to some of these alternative mRNAs and the identification and functional characterization of a promoter region upstream of it. *ACVR1* transcript, assessed by quantitative PCR after treatment with inhibitors of transcription, appeared unstable. Functional analysis of the 3′ UTR region of the *ACVR1* gene by luciferase reporter assays revealed a negative role in regulating its expression. As our in silico analysis suggested that several putative binding sites for miRNA were present in the *ACVR1* 3′ UTR region, we selected 3 of those, mir148b, mir365 and mir26a, for our experimental work. Here, we show experiments that demonstrate negative regulation of *ACVR1* expression by mir148b and mir365, as assessed by RT-qPCR on the endogenous mRNA and by transfection of pre-miR miRNA precursor molecules in combination with the *ACVR1* 3′ UTR reporter construct. With the same experimental procedures we found that mir26a could upregulate *ACVR1* expression, probably by interfering with the function of an ARE module adjacent to the mir26a binding site. Taken together, our results highlight the complexity of transcriptional and posttranscriptional regulation of *ACVR1* gene expression.

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A005_2012

AAV-Mediated Gene Therapy in Animal Models of Autosomal Dominant and X-Linked Retinitis Pigmentosa

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Background: Retinitis pigmentosa (RP) is a blinding disease affecting approximately 1 in 4000 people. We focused on developing gene-based therapy for dominantly inherited and for X-linked retinitis pigmentosa (XLRP). Mutations in 18 genes cause autosomal dominant retinitis pigmentosa (ADRP), though mutations in *RHO*, the gene for rhodopsin, are responsible for about 30% of cases. While delivery of a wild-type copy gene is often sufficient for therapy of a recessive disease, treatment of dominantly inherited disorders may require silencing of the mutant allele in addition to gene augmentation. **Methods:** For the study of ADRP we used rat and mouse models containing mutant *RHO* transgene. For XLRP, we employed 2 canine models with mutations in the *RPGR-ORF15* gene, associated with the most common form of human XLRP. We used adeno-associated virus (AAV) for gene delivery by subretinal injections. Electroretinography (ERG) and optical coherence tomography (OCT) were used to assess retinal function and structure in living animals, and microscopy was used to assess retinal preservation in fixed tissues. **Results:** We tested a combination of siRNA and resistant *RHO* cDNA in a mouse model of ADRP and observed significant protection of the retina for at least 9 months, as demonstrated by preservation of ERG response and of photoreceptor cells. In addition to this RNA replacement approach, we determined that suppression of the unfolded protein response (UPR) by gene delivery of the molecular chaperone Grp78 significantly retards retinal degeneration in a rat model of ADRP. In the canine models of XLRP, AAV-delivery of human *RPGR-ORF15* cDNA preserved rod and cone photoreceptors in the region of the retina transduced by the virus. ERG amplitudes were increased in treated eyes compared to control eyes. **Conclusions:** There is currently no effective treatment for RP. The siRNAs and ribozymes we tested for ADRP also target human *RHO*, so that the RNA replacement vectors might be useful for human gene therapy. Since mutations in *RPGR-ORF15* typically lead to early onset blindness, developing gene therapy for children with XLRP is compelling.

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A006_2012

Development of Minicircle-DNA Vectors as Non-Viral Liver-Directed Gene Therapy for Hepatic Diseases

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The liver is a potential target for transgene delivery and expression for gene therapy of hepatic and various metabolic diseases, including amino acid metabolism or urea cycle disorders.

We have previously reported long-term correction of hyperphenylalaninemia in the PKU mouse model, C57Bl/6-Pahenu2, after liver-directed gene transfer with recombinant adeno-associated viral (AAV) vectors. However, questions of expression stability, treatment toxicity, potential for insertional mutagenesis, and safety required for targeting newborn and pediatric patients for potential life-long treatment remain a risk for virus-dependent approaches. Currently, we are developing and evaluating a highly efficient non-viral gene transfer method by targeting the murine liver as a potential alternative gene-therapeutic approach. Here, we report the use of the minicircle (MC) technology for the gene therapy in the PKU mouse model. Our MC-DNA vectors contain a liver-specific promoter, various mouse phenylalanine hydroxylase (mPah) transgene expression cassettes and bovine growth hormone polyA. Delivery was mediated by hydrodynamic tail vein (HTV) injection as a liver-targeted approach, and our data showed that vectors were exclusively delivered to the liver. Subsequently, blood phenylalanine (Phe) levels normalized in PKU treated mice injected with MC-DNA containing codon-optimized mPah in a dose-dependent manner for several weeks compared to the mice injected with MC-DNA containing non-codon-optimized mPah (on-going experiment). Upon sacrificing the PKU treated mice, PAH enzyme activity was found to be elevated in liver. In summary, MC gene delivery for maximizing safety and sustained gene expression is a potential new approach for PKU treatment.

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A007_2012

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

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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. Our purpose is to develop gene therapy for RAG1-SCID patients lacking a suitable bone marrow (BM) donor. As a preclinical model for RAG1-SCID, we used Rag1$^{-/-}$ mice and lentiviral SIN vectors harboring different internal (promoter) elements, namely EF1 alpha, short form (EFS), SFFV or enhancer-less ubiquitous chromatin opening element (UCOE), to deliver native or codon-optimized human RAG1 (coRAG1) sequences. Treatment with UCOE.coRAG1 and SFFV.coRAG1 resulted in the appearance of B and T cells in peripheral blood, and developing B and T cells were detected in central lymphoid organs. Serum Ig levels and Ig and TCR Vβ gene-segment usage was comparable to wild-type (WT) controls, indicating that RAG-mediated rearrangement took place. Remarkably, relatively low frequencies of B cells produced WT levels of serum Ig in treated Rag1$^{-/-}$ mice, whereas T-cell numbers often came close to WT levels. Upon stimulation of the TCR, corrected spleen cells proliferated and produced IL-2 and IFNγ. In 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. Comparing the native and codon-optimized RAG1 vectors in vivo, the vector copy number (VCN) found in BM 19 weeks after transduction is 3- to 13-fold higher in animals that received cells transduced with the native RAG1 vector. In contrast, the RAG1 expression in these SFFV.RAG1-treated mice was 2.5-fold lower than in the SFFV.coRAG1-treated mice. On a per-copy basis, this resulted in an 18-fold higher transgene expression. 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 is promising for clinical application. To conclude, fine-tuning the use of promoters or promoter-like elements in combination with the codon-optimization of the RAG1 coding sequence has brought us closer to clinical application of lentivirus-based gene therapy for RAG-SCID patients.

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A009_2012

Progress toward the Clinical Application of Autologous Induced Pluripotent Stem Cells and Gene Repair Therapy for Treatment of Familial Hypercholesterolemia

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Stem cell transplantation has been proposed as an attractive alternative approach to restore liver mass and function. Recent progress has been reported on the generation of induced pluripotent stem cells (iPSC) from somatic cells. The production of autologous cell therapies would avoid immune rejection and enable correction of gene defects prior to cell transplantation. Here, we show for the first time reprogramming of diseased human hepatocytes (familial

hypercholesterolemia, FH) to pluripotency using a single multicistronic and excisable lentivector expressing the 4 transcription factors, Oct4, Klf4, Sox2, and cMyc. LDL uptake was restored by the transduction of a lentivector encoding the human LDL receptor under the control of a liver-specific promoter. Karyotype analysis of these cells did not show any gross genomic differences between original and reprogrammed cells. Examination of the methylome showed that iPSC exhibited a methylation pattern similar to the cells they were reprogrammed from. We then established a hepatocyte differentiation protocol under well-defined culture conditions alternating between normal and hypoxic O_2 concentrations with a cocktail of hepatocyte-specific growth factors. FH hepatocyte-derived iPSC appear indistinguishable from hESC with respect to colony morphology, growth properties, expression of pluripotency-associated transcription factors and surface markers, and in their differentiation potential via embryoid body formation and teratoma assays. These cells are able to directly differentiate into definitive endoderm, hepatic progenitors, and mature hepatocytes capable of restoring the missing metabolic function by transgenesis. Moreover, iPSC differentiation into mature hepatocytes was more efficient than with iPSC derived from fibroblasts. The development of disease-corrected hepatocyte-derived human iPSC lines will provide a foundation for studying the safety, efficacy and clinical potential of differentially-derived human iPSC for cell therapy. These results will have implications for the treatment of human liver diseases, via auto-transplantation of genetically modified hepatocytes, potentially avoiding liver transplantation and lifelong immunosuppression. For the study of liver disease pathogenesis, this technology also provides a potentially unlimited reservoir of cells from genetically corrected liver-specific iPSC.

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A014_2012

Tissue Engineering Strategy for the Repair of Congenital Diaphragmatic Hernia

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Congenital diaphragmatic hernia (CDH) is a severe congenital diaphragm malformation where abdominal content ascents into the thorax and compromises lung development in utero. By antenatal temporary positioning of a balloon in the fetal trachea respiratory failures are reduced resulting in improved survival of severely affected patients. However, their clinical outcome will rely on successful postnatal repair or reconstruction of the diaphragm. Today, large defects are treated by placing acellular biological prosthetic or synthetic materials which are either too fragile or unable to adapt in size and often need to be replaced. Amniocentesis-derived and thus ethically unproblematic mesenchymal amniocytes have been studied for the repair of diaphragmatic defects in an ovine model. The lacking vascular supply in the middle of the graft causing extensive cell death limits such an approach. Here, we present strategies to form mechanically preconditioned and vascularized tissue-engineered diaphragmic constructs from patients' own stem cells. Biomimetic materials, fibroblasts, muscle cells, endothelial cells, or amniotic fluid-derived stem cells (AFCS) will be assembled by recently established layer-by-layer deposition. Such prevascularized constructs will be mechanically stretched and thus preconditioned on a cell culture-adapted custom-made biaxial loading device. We will present data on 3D-positioning of cells in growth factor presenting biomaterials, which is required for the assembly of initial vascular structures. In order to allow site-specific stimulation of cells, growth factor immobilization strategies based on affinity interactions or covalent tethering will be shown. Additionally, initial data on the isolation, culture and phenotypic characterization of amniotic fluid cells as well as the design of our biaxial loading device are presented. Next, by rigorous phenotypic and functional characterization of individual AFCS, we will explore their potential to generate muscle, tendon or blood vessels. Well-defined subpopulations will be coaxed in engineered biomaterials and directed towards tendon, muscle, or vessel tissue. Mechanical preconditioning with cyclic loading regimen will be performed on muscle or tendon constructs. Finally, tissue-engineered diaphragms will be assembled and preconditioned to form tailor-made constructs that can be transplanted, are readily integrated and can bear up to 1 MPa of load.

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Diagnostics

B001_2012

LysoGb3 and S1P in Fabry Disease: Is There a Common Story?

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Cardiovascular Fabry disease patients manifest left ventricular hypertrophy and increased intima-media thickness. The left ventricular hypertrophy correlates with common carotid intima-media thickness. The major substrate of alpha-galactosidase A is globotriaosylceramide (Gb3), which accumulates within the tissues and organs of Fabry patients. Recently we have identified and proposed sphingosine-1 phosphate (S1P) as a growth promoting factor at the origin of cardiac and vascular abnormalities in this disease. In order to provide evidence for possible relation between S1P and Gb3, we have performed cellular immunolabeling of both S1P and Gb3 in Fabry patient and control healthy subject fibroblasts. The labeling intensities showed that both S1P and Gb3 were elevated in Fabry fibroblasts. When the labeling intensities were normalized to cell surface area, only Gb3 preserves high level labeling in Fabry patient fibroblasts. This indicates a different cellular dynamic release behavior between Gb3 and S1P. Moreover, globosphingosine (LysoGb3) was proposed as a factor involved in cardiovascular hypertrophy, which similarly to S1P induced vas-

cular smooth muscle cells (VSMC) proliferation. Based on the common structural features between S1P and LysoGb3, we hypothesized that S1P is generated from LysoGb3 that subsequently induces VSMC proliferation. Gb3 has no effect on VSMC proliferation. In contrast, LysoGb3 and S1P induced VSMC proliferation with optimum at 500 nM and 1 μM concentrations. When using S1P1 receptor antagonist W146 at 10 μM, the induced VSMC proliferation was inhibited in both cases. Our data highly suggest that S1P comparatively to Gb3 is rapidly released to extracellular milieu, Gb3 somehow generates LysoGb3 which in turn generates S1P that induces VSMC proliferation.

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B002_2012

European Registry and Network for Intoxication Type Metabolic Diseases (E-IMD)

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Background: Patients with organic acidurias (OADs) and urea cycle defects (UCDs) have an enormous need for improved medical awareness, optimization of the diagnostic process and therapy, and improved networking between healthcare professionals and patients. **Methods:** An initiative called the 'European registry and network for Intoxication type Metabolic Diseases (E-IMD)', funded by the European Commission through D.G. Sanco, started in January 2011. E-IMD aims to promote health for patients with OADs and UCDs. **Results:** E-IMD already has 49 partners from 20 countries linking healthcare professionals, patient representatives, industry and government authorities within Europe, Canada, the US, and Australia. E-IMD will continue to expand its network by inviting new members. The registry (https://www.eimd-registry) was launched in August 2011. Since then more than 130 patients have been registered. It is expected to collect data on at least 600 individuals with an OAD or UCD over the next 3 years. A website (www.e-imd.org) providing information brochures for patients, their families, and healthcare professionals in their own language was launched in August 2011. Evidence-based diagnostic and management protocols which are developed by the E-IMD consortium will also be disseminated via this website. **Conclusion:** The new network will improve access to rapid diagnosis and care for patients, will improve the knowledge base of OADs and UCDs, and will empower patients and patient organizations by providing a network and better access to expert advice and knowledge.

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B003_2012

Mutational Spectrum of Smith-Lemli-Opitz Syndrome Patients in Hungary

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Introduction: Smith-Lemli-Opitz (SLO) syndrome is a severe monogenic autosomal recessive syndrome associated with mental retardation and multiple congenital anomalies. SLO is caused by mutations in the 7-sterol reductase *(DHCR7)* gene. *DHCR7* mutations are usually small-scale and show a large inter-ethnic variability. Our goal was to set up a diagnostic scheme that includes both biochemical and molecular genetic methodology to diagnose SLO and to detect mutations responsible for SLO in Hungarian patients. **Patients and Methods:** Eleven Hungarian patients were analyzed. 7-Dehydrocholesterol (7-DHC) and cholesterol levels in the serum were measured using a UV spectrophotometry method and using an enzymatic colorimetric method, respectively. For mutation detection, the entire coding region of *DHCR7* was amplified and sequenced, and in 1 case the promoter, the non-coding exons and 3′ regions were also analyzed. **Results:** All patients had elevated 7-DHC levels (reference range <0.15 mg/l), ranging from 71.4 to 300.0 mg/l. Cholesterol levels were generally low (between 0.3 and 2.7 mmol/l). The cholesterol/7-DHC ratio was abnormal in all cases. Altogether, 10 different mutations were found, 1 splicing, 1 nonsense and 8 missense mutations. The detected alterations are known to be causative, except for the previously unidentified c.374A>G (p.Y125C) which was inherited in trans with a known mutation. The c.374A>G (p.Y125C) mutation is located in a phylogenetically conservative position. The closest known pathogenic mutations affect amino acid positions 119 and 138. Nine patients were compound heterozygous for 2 causative mutations, while 1 patient was homozygous for a null allele. In 1 patient, only one of the causative mutations could be identified. **Conclusions:** Using biochemical and molecular genetic methods, the molecular background of 10 Hungarian SLO patients could be established. Identification of mutations on 21 of 22 *DHCR7* mutant alleles was successful. In addition to the known missense, nonsense and splicing mutations, a novel, most likely pathogenic mutation was identified.

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B005_2012

Sanger Sequencing and MLPA Applied to Orphan Disease-Associated Genes

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Introduction: Meaningful diagnosis of orphan diseases requires knowledge of the underlying molecular lesion. This will not only support the clinical diagnosis (and thus the treatment),

but also help parents in taking decisions regarding future offspring. In the current study, we retrospectively evaluated the results of our genes-on-demand Sanger sequencing and MLPA service for orphan disease genes. **Methods:** Primers amplifying the exons and the adjacent intronic sequences of each gene were designed with ExonPrimer and Primer 3 and checked for specificity and polymorphisms with SNPCheck. PCR conditions were established with wildtype DNA and then applied to patient DNA, followed by Sanger sequencing. Sequences were analyzed with SeqScape or SeqPatient, and positive results were confirmed by an independent PCR. In case a gene-specific probe-kit was available, MLPA was performed, with identified deletions being confirmed by GAP-PCR. The current survey is based on written reports of these screenings; it does not include thalassemia and *BRCA1/BRCA2* screenings as well as mutation-specific analyses. **Results:** Over the last 2 years, we analyzed 43 different genes in 59 patients. In 6 of these 59 patients, more than 1 gene was analyzed (e.g. *COL1A1* and *COL1A2*). The most commonly requested analysis was screening of *MECP2* (associated with RETT syndrome, 7 cases), followed by *CFTR* (cystic fibrosis, 4 cases), and *MEFV* (familial mediterranean fever, 4 cases). 23 analyses had been requested only once by December 2011. In 19 patients, disease-causing mutations could be identified, giving an overall mutation detection rate of 32%. Of the 26 mutations identified, 15 (58%) were missense mutations, 8 (31%) nonsense, and 2 (8%) splice mutations. The only deletion identified was detected by PCR failure of the X-chromosomal *XK* gene. In all genes investigated, no deletion was detected by MLPA. **Discussion:** We identified disease-causing mutations in a substantial number of patients, demonstrating that Sanger sequencing can be highly supportive in the diagnosis of orphan diseases.

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B006_2012

Structural Analysis of Saccharide-Lipid Deposits Using Chromatographic Methods in Cell Lines from Patients with Mucopolysaccharidoses

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Mucopolysaccharidoses (MPSs) are rare hereditary metabolic diseases belonging to the group of lysosomal storage diseases. They are caused by a defect in a specific enzyme involved in degradation of glycosaminoglycans (GAGs). Deficiency or lack of enzyme activity leads to accumulation of compounds that are substrates for this enzyme in lysosomes, and consequently to a gradual damage of cells, tissues and organs. Research in improving the life of patients suffering from MPS is ongoing on various therapeutic approaches. Therefore, it is important to implement an appropriate method to control the changes in the level of individual GAGs, as well as to monitor the effectiveness of the therapy, for both patients and basic knowledge, as most research on treatment begins with in vitro studies on cell cultures. Determination of the individual GAGs and monitoring of their level in cells exposed to GAG synthesis reducers or their degradation factors using high pressure liquid chromatography (HPLC) is the topic of our work. Such analysis has an application in evaluating the effects of experimentally induced changes in expression levels of biosynthetic enzymes or other specific regulatory factors. A simple procedure, requiring only standard HPLC instrumentation, involving isolation, purification and precipitation of GAGs, followed by a separation by reverse-phase HPLC that is sensitive to as little as ~100 pg of an individual disaccharide, thereby allowing analyses of >10 ng of total GAG, is handled. As for GAG isolation methods, protocol optimization was carried out by using papain and proteinase K treatment of fibroblasts. The samples were passed through DEAE-Sepharose columns, followed by their precipitation on PD-10 columns to provide total GAG preparation for disaccharide analysis by HPLC. Disaccharide analysis of particular sulphated GAGs, instead of their entire content in the sample, is a qualitative approach to quantify individual GAGs in MPS and HDFa (human dermal adult) fibroblasts in contrast to untreated cultures. So far, as a result of the experiments, we determined the suitability of the application of HPLC to monitor the effectiveness of therapeutic agents used in in vitro studies.

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B007_2012

DORA (Doenças Raras) Project: A Proposal for an Integrated Management of Rare Diseases in the State of Sao Paulo, Brazil

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The State of Sao Paulo (Brazil) has a population of 41 million inhabitants with 600,000 live births/year. It has been experiencing significant advances in the control of diseases related to malnutrition-infection binomial, but the infant mortality rates are still high (11.9/1,000 live births in 2010). 21% of these deaths are due to congenital malformations and rare diseases, which now emerge as major focus of attention within the context of the State's public health system. The purpose of the present communication is to introduce the DORA project, a joint effort between the Children's Hospital (University of Sao Paulo Medical School) and the Sao Paulo Secretary of State for Health, in order to organize early diagnosis and integrated care of congenital malformations and rare diseases in the State of Sao Paulo. The following actions are currently under way: (1) Creation of a network of the university hospitals and outpatient clinics for early diagnosis and protocol-based treatment. (2) Establishment of a 'warning signs' list and protocol-based clinical management (as a basis for future cohort studies) for 1–2 diseases in each one of the following areas of interest: metabolic diseases, neuromuscular diseases, growth and sexual differentiation diseases, primary immunodeficiency, kid-

ney and urinary tract disorders, ophthalmology, orthopedics, pulmonology, HEENT, hematology, chromosomal anomalies and complex malformations and cardiac malformations. (3) Creation of an integrated computer system with the following interfaces: (A) Non-health professional homepage with an A–Z list of topics on rare diseases, a list of available services for diagnosis and follow-up, a list of patient advocacy organizations; (B) a homepage for health professionals with an A–Z list of topics on rare diseases, a list of available services for diagnosis and patient follow-up; links to educational videos presenting the warning signs for each group of clinical conditions, a link to consult with a specialist before referring a difficult case and a link for the university hospitals network participants to be used for cohort studies data management. It is our estimate that the DORA project will be launched in the second semester of 2012 after completion of the integrated computer system which will enable dissemination of concepts of the warning signs for primary care doctors and cohort-related data management by the network participants.

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B008_2012

Consanguinity as a Means to Identify Pathogenic Recessive Mutations

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Consanguinity and inbreeding increases the sharing of alleles among individuals. We have initiated a project to study recessive phenotypes in consanguinity families, in order to identify the functional genomic variation responsible for the disease. Any phenotype and family history compatible with autosomal recessive inheritance is a candidate for participation in the study, and 42 families of different ethnic background have already been collected. From each family, blood DNA from the patient(s), all unaffected siblings and the parents is extracted. Samples from one or more of the affected individuals per family are examined by aCGH 400K for the detection of large homozygous deletions. The samples of all the family members are genotyped with a dense SNP array in order to identify Runs of Homozygosity (ROH), allowing the definition of chromosomal regions likely to contain the responsible genes. Exome sequencing is performed in one of the affected individuals of each family. Variants are called using publicly available tools and filtered according to polymorphic SNVs deposited in public databases and predicted pathogenicity. 12 families have been completely analyzed using this approach. Causative variations of known disease genes have been identified in 2 families (*VLDLR* gene, causing disequilibrium syndrome and *FKTN* gene, causing Fukuyama muscular dystrophy). In 5 additional families candidate genes have been identified. Consanguineous families provide an opportunity to identify genes responsible for recessive phenotypes and rapidly fill in the space of genotype-phenotype links.

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B009_2012

Targeted Next Generation Sequencing for Clinical Diagnostics of Patients with Myeloproliferative Neoplasms

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Statement of Purpose: Myeloproliferative neoplasms (MPNs) are a group of diseases characterized by aberrant proliferation of the myeloid, erythroid and/or megakaryocytic lineages. A number of gene mutations have been described that can initiate or advance MPN when acquired as a somatic event in hematopoietic stem cells (e.g. mutations in the genes *JAK2*, *MPL*, or *TET2*). However, as the list of mutations grows larger, there is a need for improved diagnostic methods in order to genotype a patient for diagnostic, prognostic and treatment purposes. Here we describe a clinically applicable method for mutation screening using multiplexed enrichment of desired genes followed by next generation sequencing (NGS). **Methods:** DNA from 48 MPN patients was individually fragmented and barcoded using indexed adapters. The DNAs were pooled and enriched using an Agilent SureSelect custom design. The custom design covers all genes where mutations have been reported in MPN, as well as 100 additional genes involved in hematopoietic signaling. After enrichment, samples were sequenced using Illumina HiSeq2000. All patients were analyzed in duplicates using separate PCR reactions and enrichments to reduce false positive results. **Results:** The average read coverage of the regions of interest was 474× and 411× for the duplicate runs of the first 48 patients, respectively. The amount of exons covered by >20 reads was 95.9% and 95.6% and >100 reads 90.5% and 89.5%, respectively. Previously observed mutations in these 48 patients were used to validate the mutations generated by NGS of which 33/34 *JAK2*-V617F mutations, 3/3 *JAK2* exon 12 deletions and 4/4 *TET2* alterations were detected. The *JAK2*-V617F mutation not detected using NGS could only be found in 1 of the 2 duplicate reads, however, this mutation was present in <1% of the

cells as determined using allele-specific PCR. In addition, 1 novel *JAK2* mutation, 12 additional mutations in *TET2* (9 missense and 3 nonsense), 6 *DNMT3A* (3 R882H, 2 other missense and 1 nonsense) and 2 *IDH1* (R132H) mutations were discovered. Also 90 mutations in genes with no previously reported involvement in MPN were found. Analysis of the somatic nature of these mutations is currently underway. **Conclusion:** Multiplexed enrichment and NGS was found to be a cost efficient and reliable tool for a cohort-based screening of MPN patients. The method can be applied to any disorder where multiple genes are to be screened for diagnostic purposes with high sensitivity.

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B010_2012

FBN1, *TGFBR1*, *TGFBR2*, and *SLC2A10* Mutation Analyses in Patients with Suspected Marfan Syndrome: A Swiss Study

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Marfan syndrome (MFS) is caused by *FBN1* mutations in the majority of cases. Many of the features of MFS show overlap with related aortic disorders, such as Loeys-Dietz syndrome (LDS), familial thoracic aortic aneurysms and dissections (TAAD), and arterial tortuosity syndrome (ATS). In patients with suspected MFS, *FBN1* genetic testing detects only ~80% of mutations. This may be due to technical limitations of currently used PCR-based screening methods and/or because the disease-causing mutation occurs in a different gene. We have investigated the impact of these possibilities. In a cohort of unrelated individuals with suspected MFS in whom previous analysis of *FBN1* revealed no mutation, we have sequenced the genes *TGFBR1*, *TGFBR2*, and *SLC2A10*. We have also screened for large deletions/duplications by multiplex ligation-dependent probe amplification (MLPA). The pathogenic impact of novel sequence variants was assessed by in silico predictions and/or RT-PCR and segregation analyses. The breakpoints of large deletions identified by MLPA were narrowed down by using microarrays. In patients with suspected MFS who finally could be diagnosed with LDS and TAAD, we identified heterozygous *TGFBR1* or *TGFBR2* nucleotide substitutions and in 1 ATS patient a homozygous *SLC2A10* nonsense mutation. The deleterious alleles occurred de novo or segregated with the disease in the families, indicating a causative association between the sequence variants and clinical phenotypes. Neither a *TGFBR1*- nor a *TGFBR2*-specific phenotype could be detected. In 2 patients, MLPA revealed large genomic rearrangements affecting *FBN1*. Our data demonstrate that *TGFBR1* mutations are associated not only with LDS but also with TAAD, and that true *FBN1* haploinsufficiency is sufficient to cause MFS.

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B011_2012

Evaluation of Exome Sequencing with Different Types of Sequence Variations in Genes Associated with Aortic Diseases

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Whole-exome sequencing is a combination of ultra-high-throughput next generation sequencing and the state-of-the-art enrichment of all known human protein-coding exons and flanking canonical splice sites. In addition to qualitative analyses, which can detect point mutations and small insertions/deletions, whole-exome sequencing data can also be used for quantitative sequence analysis in order to detect large insertions and deletions (copy number variations). In this study, we have evaluated the qualitative and quantitative properties (i.e. mutation detection rate) of exome sequencing for different mutation types and genes. These genes are associated with syndromic forms of rare aortic diseases, such as Marfan syndrome *(FBN1)*, Loeys-Dietz syndrome *(TGFBR1* and *TGFBR2)*, and Ehlers-Danlos syndrome vascular type *(COL3A1)*, or with non-syndromic forms such as familial thoracic aortic aneurysms *(ACTA2, MYH11*, and *MYLK)*. For this evaluation, DNA samples with known point mutations and small deletions/duplications detected by Sanger sequencing as well as large deletions/duplications detected by MLPA were used as template in exome sequencing. In a first step, we applied Agilent's in solution sequence capturing of all coding exons and flanking intronic sequences and performed next generation sequencing using a SOLiD4 platform. Exome sequencing data visualized by the Integrative Genomics Viewer (IGV) revealed that the mutation detection rate of the used exome sequencing method was lower than that of Sanger sequencing and MLPA, varying between mutation types and genes. Whereas point mutations were successfully detected in enriched exons with sufficient read-coverage depth, the used exome sequencing protocol needs to be improved for the detection of small deletions and duplications/insertions as well as for the more balanced capturing (enrichment) of exons.

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B012_2012

Hemizygous Deletion Leading to True Haploinsufficiency of *COL3A1* Causes Aortic Dissection

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Aortic dilatation/dissection (AD) can occur spontaneously, non-syndromic or in association with genetic syndromes such as Marfan syndrome (MFS) caused by *FBN1* mutations, Loeys-Dietz syndrome caused by *TGFBR1* or *TGFBR2* mutations, and vascular Ehlers-Danlos syndrome (EDS IV) caused by *COL3A1* mutations. Although mutations in *FBN1*, *TGFBR1* and *TGFBR2* account for the majority of AD cases referred to us, we have encountered negative genetic testing results in a large group of patients, suggesting the involvement of other genes, e.g. *COL3A1*, *ACTA2* or *MYH11*. In this study, we have assessed the impact of *COL3A1* mutations in patients with suspected MFS in whom mutation screening in *FBN1*, *TGFBR1* and *TGFBR2* revealed no disease-causing sequence variation. MLPA analysis of 100 unrelated patients identified hemizygous deletion of the entire *COL3A1* gene in 1 patient with abdominal AD. Subsequent microarray analyses and sequencing of breakpoints revealed the deletion size of 3,408,306 bp. Furthermore, gDNA sequencing revealed *COL3A1* sequence variants in some of our patients. Our data not only emphasize the importance of screening for *COL3A1* mutations in comprehensive genetic testing of AD patients with suspected MFS not fulfilling the Ghent criteria, but also extend the molecular etiology of EDS IV by providing evidence for true haploinsufficiency of *COL3A1*.

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B013_2012

Bone Marrow Alteration in Patients with Type 1 Gaucher Disease on Enzyme Replacement Therapy

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Purpose: To analyze changes in the bone marrow in patients with Type 1 Gaucher disease and correlate the results with genotype and laboratory findings. **Materials and Methods:** MRIs of the lower extremities performed between 1996 and 2011 to evaluate the effect of enzyme replacement therapy (ERT) on bone marrow infiltration by Gaucher disease were reviewed. Signal changes were recorded before initiating ERT and followed annually or biannually. The marrow changes were classified as: decreased, scattered or confluent, signal on T1 weighted imaging (WI); increased or unchanged on STIR imaging. The extent of the marrow involvement was also evaluated. The patient's genotype was recorded. The changes were correlated with laboratory findings. **Results:** 285 patients' MRIs were examined. 52 patients on ERT showed improvement in the marrow infiltration over the study period. These patients have a variety of genotypes, but the majority, 35/52 (67%), have the milder N370S/N370S genotype. In general, bone marrow improvement is a slow process that does not manifest immediately with ERT but occurs over the course of several years. **Conclusion:** Bone marrow infiltration in patients with Type 1 Gaucher disease does respond to ERT but generally takes many years to manifest on MRI. Although those that responded were of several different genotypes, the majority had the more benign N370S/N370S. Surveillance MRI is essential for clinical decision making concerning ERT and dosing.

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B014_2012

Prevalence of Gallstones in Patients with Type 1 Gaucher Disease

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Purpose: To compare the prevalence of gallstones in patients with Type 1 Gaucher disease with that of the general population. **Material and Methods:** The Comprehensive Gaucher Disease Treatment Center at our institution follows approximately 300 patients with Type 1 Gaucher Disease. Each patient is followed with abdominal MRI or CT scans to evaluate visceral manifestations of Gaucher disease. These imaging studies from 1994 to present were reviewed to determine if each patient had gallstones or had undergone cholecystectomy. The patient's sex and age at the time of the first imaging study that demonstrated gallstones or gallbladder surgery were recorded. Comparison was made to the generally accepted 10% prevalence of gallstones in the general population. **Results:** 271 patients had studies to evaluate; 131 males and 140 females, age range 7–96 years. 54 patients had gallstones or cholecystectomy yielding a prevalence of 20% (54/271). 40 patients (19 male, 21 female) had gallstones. Of these patients, 15 (5.5%) also had undergone splenectomy. 14 patients (5 male, 9 female) had undergone cholecystectomy. Of these patients, 7 (2.6%) also had undergone splenectomy. The age group most affected in males was 61–70 years, followed by 51–60 years; in females the most common age group was 51–60 years, followed by 31–40 years. **Conclusion:** The prevalence of gallstones in Type 1 Gaucher patients is significantly higher than that of the general population. Females are more commonly affected than males and appear to manifest signs or symptoms at a younger age. The high prevalence of gallstones is likely due to the abnormal biliary lipid secretion that can be a manifestation of Gaucher disease. **Clinical Relevance:** Gallstone disease has increased prevalence in Type 1 Gaucher Disease patients but splenectomy does not appear to be a significant risk factor.

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B015_2012

Molecular Analysis of Polish Patients with Epidermolysis Bullosa Simplex

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Epidermolysis bullosa (EB) is a group of genodermatoses characterized by spontaneous or mechanically induced blister formation. Depending on the level of epidermis-dermis separation during bullae formation, 4 EB types can be distinguished: simplex (SEB), junctional (JEB), dystrophic (DEB) and Kindler syndrome (KS). Clinical symptoms vary between those types and about 30 subtypes have been described in EB. SEB is recognized when blisters form in basal or suprabasal layers of epidermis. There are about 12 subtypes in SEB, of which the 3 commonest, localized SEB (previously known as Weber-Cockayne), Dowling-Meara SEB and other generalized SEB (previously Koebner), are autosomal dominant and caused by mutations in *KRT5* and *KRT14* genes. However, mutations in *KRT14* are also found in rare autosomal recessive SEB subtype and *KRT5* in some other sporadic variants. The aim of the study was to investigate the spectrum of mutations in Polish SEB patients. 34 probands (10 localized SEB, 2 Dowling-Meara SEB, 5 other generalized SEB, 17 undefined) diagnosed on the basis of clinical symptoms or immunofluorescence mapping were investigated in the study. DNA was isolated from leukocytes and analyzed by direct sequencing of coding regions of *KRT5* and *KRT14*. In this group of patients, we found 9 distinct variants in *KRT5* and 7 in *KRT14*, including overall 7 changes unreported before. In 17/34 (50%) cases full genotype was established; in 5 cases no mutations in *KRT5* and *KRT14* genes were identified. In the remaining 12 patients molecular analysis has not been completed yet. Mutations p.Val186Met and p.Glu170Lys in *KRT5* were found in at least 2 distinct families each. In 1 patient with mild SEB and her affected brother, 2 variants in *KRT5* gene, p.Val143Ala and p.Glu170Lys, were identified. Both parents of these patients are carriers and seemed to be unaffected. To our knowledge, only few patients with compound heterozygosity in *KRT5* have been reported previously. In conclusion, our preliminary results broaden the knowledge about pathogenesis and epidemiology of SEB and also have practical impact on preparing the Polish population-specific molecular diagnostics scheme.

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B017_2012

The Ratio of Urinary Pyridinoline to Deoxypyridinoline Crosslinks – A Promising Diagnostic Tool in Osteogenesis Imperfecta

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Urinary pyridinoline crosslinks, hydroxylysyl-pyridinoline (HP, or pyridinoline PYD) and lysyl-pyridinoline (LP, or deoxypyridinoline DPD) are well characterized markers for bone resorption and collagen degradation and proven diagnostic tools for genetic disorders of collagen metabolism such as Ehlers-Danlos syndrome (EDS) VIA, SCD-EDS and Bruck syndrome. Osteogenesis imperfecta (OI) is a heterogeneous genetic disorder characterized by increased susceptibility to fractures. The majority of OI is inherited as an autosomal dominant trait caused by mutations in *COL1A1* and *COL1A2*. A small proportion of OI is inherited in an autosomal recessive manner due to mutations in 8 different genes encoding proteins of the prolyl 3-hydroxylation complex *(CRTAP, LEPRE1, PPIB)*, collagen chaperones *(SERPINH1, FKBP10)*, a proteinase *(BMP1/mTLD)* involved in processing of the procollagen I C-terminal propeptide, a transcription factor *(SP7/OSX)* assumed to regulate the differentiation of preosteoblasts to osteoblasts, and *SERPINF1*, a secreted glycoprotein of the serpin superfamily. The aim of this study was to evaluate the ratio of total urinary pyridinolines LP/HP (or: DPD/PYD) as a non-invasive, reliable and cost effective screening tool in individual OI patients, prior to collagen biochemical and/or molecular genetic analyses. We analyzed spot urines of controls and OI patients of known genetic background, with defects in *LEPRE1, CRTAP, SP7/OSX* and *SERPINF1*, as well as heterozygous carriers and 20 patients with dominant mutations in *COL1A1* or *COL1A2*. Compared to controls (0.20 ± 0.03, n = 325), we found markedly decreased LP/HP ratios in OI caused by mutations in *LEPRE1* (mean: 0.078, n = 3), *CRTAP* (mean: 0.105, n = 3) and *SP7/OSX* (0.086), and slightly decreased LP/HP ratios in heterozygous carriers for mutations in *CRTAP* (mean: 0.172, n = 2) and *SP7/OSX* (mean: 0.128, n = 2). We found normal LP/HP ratios for mutations in *COL1A1/COL1A2* (mean: 0.20; n = 20), and *SERPINF1* (mean: 0.195, n = 2), and in the heterozygous carriers for a *LEPRE1* defect (mean: 0.2, n = 2). Thus, LP/HP ratios have the potential to detect recessive forms of OI caused by mutations in the genes *LEPRE1, CRTAP* and *SP7/OSX*, thereby improving the diagnostic efficacy and reducing the costs of molecular genetic investigations. Similarly, we expect decreased LP/HP ratios in OI cases caused by mutations in *PPIB*. With this report, we hope to attract more cases of OI with a known genetic defect in order to statistically validate this preliminary study.

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Therapeutic Applications

C001_2012

Oral L-Serine Supplementation as a Therapy in Hereditary Sensory Autonomic Neuropathy Type 1 (HSAN1)

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Background: Sphingolipids are commonly formed from the precursors L-serine and palmitoyl-CoA – a reaction which is catalyzed by the serine-palmitoyltransferase (SPT). Several SPT missense mutations are associated with the inherited sensory neuropathy HSAN1 – a rare axonal neuropathy which typically presents with decreased pain and temperature sensation accompanied with painless blisters and ulcers. **Results:** SPT is not strictly depending on serine but metabolizes also alanine and glycine to a certain extent. This results in the formation of 1-deoxy-sphingolipids (dSL) which lack the C1 hydroxyl group of normal sphingolipids. They are therefore not converted into complex sphingolipids nor degraded by the normal catabolic pathway. This promiscuous activity is greatly increased in HSAN1. Significantly elevated dSL levels were found in plasma from HSAN1 patients but also in plasma and PNS tissue of transgenic HSAN1 mice. Deoxy-sphingolipids were shown to be neurotoxic and to induce neurite retraction in cultured primary neurons. Interestingly, dSL formation is suppressed at elevated L-serine levels. HSAN1 mice which received an L-serine enriched diet showed significantly reduced plasma dSL levels. On long term supplementation the mice were protected and did not develop neurological symptoms. In contrast, alanine fed mice developed severe neurological deficits already at the early age of 3 months. The positive effects of an L-serine supplementation were further corroborated in a human pilot study with 14 HSAN1 patients. The patients received an oral serine treatment (200 or 400 mg/kg/day) for 10 weeks. Within 6 weeks dSL levels decreased to normal values in both groups and increased again after the end of the trial. Despite the short duration of this study some patients reported an increase in sensation (hand tingling, increased menstrual cramps) and significant improvements in skin robustness and body hair growth. **Conclusions:** Our results showed that an elevated dSL generation is the pathological cause for HSAN1. An oral supplementation with the amino acid L-serine suppressed dSL formation and might therefore be a potential therapy in HSAN1. Initial results of a short term intervention study were positive and are currently re-evaluated in a more comprehensive clinical trial. If successful, this would be the first available therapy in HSAN1 but also the first rational treatment option for an inherited peripheral neuropathy in general.

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C002_2012

From Rationing to Rationality: An N-of-One Trial Service for Off-Label Medicines for Rare Diseases

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Background: In the Netherlands, some medicines are not reimbursed for off-label use without sufficient evidence of efficacy. Patients with rare (including genetic) diseases are disadvantaged because the burden of proof is difficult to meet. There are obstacles both for industry and academia to performing large-scale randomized, controlled trials for rare diseases. Reimbursement rules also discourage doctors from prescribing medicines off-label, even to small groups of patients. Controlled n-of-one (single-patient) trials with internal randomization (e.g. AB-BA-BA) could generate evidence on efficacy for rare, chronic conditions where the aim of treatment is symptom control. **Objective:** This project aims to initiate development of an n-of-one trial service, embedded in the Dutch health care system, for research on efficacy and safety of certain medicines with no marketing authorization for the rare diseases for which they are prescribed. **Methods and Preliminary Results:** Reimbursement problems with off-label medicines for rare neuromuscular diseases were inventoried among neuromuscular specialists and patients with neuromuscular disease in the Netherlands. A multidisciplinary expert meeting was organized to define legal, ethical and scientific preconditions for formalizing and sustaining an n-of-one trial service. The problem was widely acknowledged by stakeholders. Willingness was expressed to consider new forms of evidence for efficacy and safety of medicines for rare diseases and personalized therapies. Recommendations and preconditions for carrying out n-of-one research in a scientifically sound and socially robust manner were given. **Implications:** Regulatory authorities and insurers may accept evidence from n-of-one trials, provided that data can be aggregated and that benefit/risk ratio is considered. An n-of-one trial service can facilitate this process.

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C003_2012

Feasibility of Nonsense Mutation Readthrough as a Novel Therapeutical Approach in Propionic Acidemia

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Aminoglycosides and other compounds can promote premature termination codon (PTC) readthrough, constituting a potential therapy for patients with nonsense mutations. In a cohort of 190 propionic acidemia patients, we have identified 12 different nonsense mutations, 6 of them novel, accounting for 10% of the mutant alleles. Using an in vitro system we establish the proof-of-principle that nonsense mutations in the *PCCA* and *PCCB* genes encoding both subunits of the propionylCoA carboxylase (PCC) enzyme can be partially suppressed by aminoglycosides, with different efficiencies depending on the sequence context. To correct the metabolic defect, the amino acid incorporated at the PTC, usually Gln or Trp, should support protein function and this has been evaluated in silico and by in vitro expression analysis of the predicted missense changes, most of which retain partial activity, confirming the feasibility of the approach. In patients' fibroblasts cultured with readthrough drugs we observe a 4–50-fold increase in PCC activity, reaching up to 10–15% levels of treated control cells. The ability to partially correct nonsense *PCCA* and *PCCB* alleles represents a potential therapy or supplementary treatment for a number of propionic acidemia patients encouraging further clinical trials with readthrough drugs without toxic effects such as PTC124 or other newly developed compounds.

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C004_2012

Proteasomal Inhibition Restores Biological Function of Missense Mutated Dysferlin in Patient-Derived Muscle Cells

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Background: Dysferlin is a transmembrane-protein implicated in surface-membrane repair of muscle cells. Mutations in dysferlin cause the progressive muscular dystrophies Miyoshi-Myopathy, Limb-Girdle-Muscular-Dystrophy 2B, and distal-anterior-compartment-myopathy. Dysferlinopathies are inherited in an autosomal recessive manner, and many patients with this disease harbor missense mutations in at least 1 of their 2 pathogenic *DYSF* alleles. These patients have significantly reduced or absent dysferlin levels in skeletal muscle, suggesting that dysferlin encoded by missense alleles is rapidly degraded by the cell's quality-control system. We reasoned that missense mutated dysferlin, if salvaged from degradation, might be biologically functional. **Methods:** We used a dysferlin-deficient human myoblast culture harboring the common Arg555Trp missense allele and a *DYSF* null allele, as well as control human myoblast cultures harboring either 2 wild-type or 2 null alleles. We measured dysferlin protein and mRNA levels, resealing kinetics of laser-induced plasmalemmal wounds, myotube formation, and cellular viability after treatment of the human myoblast cultures with the proteasome inhibitors Lactacystin or Bortezomib (Velcade). **Results:** We show that endogenous Arg555Trp missense mutated dysferlin is degraded by the proteasomal system. Inhibition of the proteasome by Lactacystin or Velcade increases the levels of Arg555Trp missense mutated dysferlin. This salvaged protein is functional as it restores plasma membrane resealing in patient-derived myoblasts, and reverses their deficit in myotube formation. Bortezomib and Lactacystin did not cause cellular toxicity at the regimen used. **Conclusion:** Our results raise the possibility that inhibition of the degradation pathway of missense-mutated dysferlin could be used as a therapeutic strategy for patients harboring certain dysferlin missense mutations.

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C005_2012

Regulatory Experience with Proposed Treatments for Mendelian Disorders in the United States

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Purpose: Identification of features underlying success or failure of applications for FDA approval of proposed treatments for Mendelian disorders. **Methods:** Exhaustive review of publicly available FDA Orphan Designations and Summary Bases of Approval for Mendelian disorders, as well as our anonymized experience with an extensive but nonrandom sample of regulatory dossiers that have failed to achieve approval. **Summary:** As of 29 November 2011, there are 448 FDA designations of Orphan Status for proposed treatments of Mendelian disorders, with a skewed distribution among 121 disorders, 8 with more than 10 designations each. In descending order, designations were awarded to medium to small cap biotechnology firms (298), large pharmaceutical houses (113), unaffiliated individuals (21), academic institutions (9), disease-related charities (6), and a governmental agency. The overall-rate of marketing approval for Mendelians has been 16.5%, nearly identical to the 15.6% approval rate for all 2,520 designated orphans, the majority for oncology. However, the approval rate for Mendelians varies significantly with therapeutic class [small molecule (9.9%) or biologics (24.2%)] and the nature of the sponsor. Among the biologics designated for Mendelian disorders (215), all approvals have been for proteins (52), none for advanced therapeutics (cells, genes, inhibitory RNAs) even though the latter have received 55 Mendelian designations, one advanced therapeutic having been approved in 2010 for oncology. Mendelian orphans sponsored by large pharmaceutical companies achieved an overall approval of 34.5% vs. 11.4% for biotechs. The approval advantage of integrated pharmaceutical companies over other sponsors spanned therapeutic classes: proteins 46.3% vs. 22.6%, small molecules 23.1% vs. 7.2%. This dis-

parity accords with our observations of difficulties in demonstrating consistent manufacture and safety, as well as our corroboration of findings published by Heemstra: failures resulting from 'clinical trial design, the level of experience of the sponsor and the level of interaction with the FDA.' **Conclusions:** Although the focus of nonpharmaceutical sponsors is frequently centered on proof of concept, equal attention needs to be afforded to CMC (chemistry, manufacturing, control) safety, dose selection, and design of pivotal trials, the 4 predominant causes of failure for Mendelians, as for other indications when resource constraints preclude thorough investigation.

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C006_2012

Lessons from an Ultra-Rare Disorder: A New Insight into Glutamine Synthetase Deficiency

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Background: A defect in glutamine synthetase (GS) leads to systemic glutamine deficiency and is an ultra-rare disorder described so far in only 3 patients with severe epileptic encephalopathy. This rareness possibly points towards the indispensable role of GS, in particular since glutamine is the unique amino moiety donor for many substances including nicotinamide adenine dinucleotide (NAD+). Thus, deficiency of glutamine in itself is a major factor of this disease, but still the entire pathophysiology of GS deficiency has not been fully understood. **Materials and Methods:** Building up on the recent description of the natural course of GS deficiency in a 3-year-old patient, we performed functional studies in different cell types (in vitro in fibroblasts and lymphoblasts, in vivo in leukocytes) obtained from this single living patient affected by GS deficiency and hereby focused on NAD+ metabolism. In detail, we studied the basal NAD+ concentrations in these cells and compared them with levels after substitution of glutamine or nicotinamide. **Results:** Both in vitro and in vivo, untreated cells revealed a severe lack of NAD+. However, substitution of either glutamine or nicotinamide allowed to correct the deficiency of this abundantly needed energy metabolite. **Conclusion:** Albeit being an ultra-rare disorder with only 1 known living patient, studies presented here underline the absolute requirement of glutamine for NAD+ metabolism. This study highlights the potential of rare disorders to help elucidating basic biological processes and opening the window for novel therapeutic approaches.

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C007_2012

Beneficial Effects of Early Childhood *Helicobacter pylori* Infection on the Development of Allergic and Chronic Inflammatory Disorders

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Helicobacter pylori infection affects 50% of the world's population and is associated with chronic gastritis in all infected individuals which can lead to gastric and duodenal ulcers, MALT-lymphoma and adenocarcinoma. Recently, our lab reported that neonatal infection of mice with *H. pylori* protects from development of gastric pathology by induction of tolerance to the bacterium. This is accomplished by the capacity of *H. pylori* to induce semi-mature dendritic cells, which activate T regulatory cells. Furthermore, we were able to confirm epidemiological data demonstrating an inverse correlation between *H. pylori* infection and asthma development by showing that the T regulatory cells induced by neonatal *H. pylori* infection can protect from ovalbumin-induced asthma. Our recent experiments show that the oral administration of *H. pylori* sonicate also efficiently suppresses asthma development. Epidemiological studies have also demonstrated an inverse correlation between *H. pylori* infection and inflammatory bowel disease, major forms of which are Crohn's disease and ulcerative colitis. In a DSS-induced mouse model of colitis, mice infected in the neonatal period showed less pathology and less secretion of pro-inflammatory cytokines. Interestingly, a strategy of 'tolerizing vaccination' with *H. pylori* sonicate is almost fully protective in our mouse model of inflammatory bowel disease. We will now investigate the Treg involvement of sonicate protection by adoptive transfer experiments and will try to elucidate the bacterial factor as well as the host molecular mechanism that induces the protective effect. Our overall goal is to define preventive and therapeutic treatment strategies for asthma and inflammatory bowel disease.

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C008_2012

Neonatally Acquired Immunological Tolerance to *Helicobacter pylori* Infection Prevents Gastric Immunopathology and Protects against Asthma and Chronic Colitis

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Persistent infection with the gastric bacterial pathogen *Helicobacter pylori* causes gastritis and predisposes carriers to a high gastric cancer risk, but has also been linked to protection from allergic, chronic inflammatory and autoimmune diseases. The beneficial consequences of chronic *H. pylori* infection have been proposed to result from the pathogen's immunomodulatory prop-

erties. We have utilized mouse models of allergic airway disease induced by ovalbumin or house dust mite allergen to experimentally examine a possible inverse correlation between *H. pylori* and asthma, a chronic T-cell driven disease of the airways. *H. pylori* infection efficiently protected mice from the airway hyper-responsiveness, tissue inflammation and goblet cell metaplasia that are hallmarks of asthma, and prevented the allergen-induced pulmonary and bronchoalveolar infiltration of eosinophils, Th2 and Th17 cells. Protection against asthma was abrogated by antibiotic eradication of *H. pylori* and was most robust in neonatally infected mice, which develop peripheral tolerance to *H. pylori*. Asthma protection was further associated with impaired maturation of lung-infiltrating dendritic cells (DCs) and accumulation of highly suppressive regulatory T-cells in the lungs. Systemic Treg depletion abolished asthma protection; conversely, the adoptive transfer of purified Treg populations was sufficient to transfer protection from infected donors to uninfected recipients. Our results thus provide experimental evidence for a beneficial effect of *H. pylori* colonization on the development of allergen-induced asthma. We have further investigated the involvement of DCs in tolerance induction to *H. pylori*. Infection of DCs with *H. pylori* reprograms the cells towards a tolerance-promoting, semi-mature state. The experimental depletion of DCs breaks *H. pylori*-specific, neonatally acquired tolerance and results in improved control of the infection, but also in more severe immunopathology. Interestingly, 'tolerizing' vaccination with *Helicobacter* sonicate is as efficient at preventing asthma as live infection. Similarly, vaccination or live infection efficiently prevents chronic colitis in experimental models of the disease. Overall, our results suggest that *H. pylori* reprograms DCs in favor of tolerance over immunity to maintain persistence and that this systemic immunomodulation protects against asthma and other chronic T-cell driven immunopathologies.

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C009_2012

Comparing R&D Incentives for Neglected Diseases

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Neglected diseases are typically characterized as those for which adequate drug treatment is lacking, and the potential for return on investment in research and development (R&D) to produce new therapies is too small to stimulate companies to invest significant resources in the field. Examples include infectious diseases that predominantly affect the developing world, such as African trypanosomiasis and schistosomiasis. Various incentive mechanisms have been associated with such initiatives. Broadly speaking, these can be classified either as 'push' or 'pull' programs. Push programs have a direct impact on R&D expenditures, supporting drug discovery, and often take the form of upfront research grants, from public institutions or charities to pharmaceutical firms. Pull incentives instead stimulate research effort indirectly, by enhancing the revenue potential and/or lowering delivery costs. Examples include differential pricing, advanced market commitments (AMC) and prize mechanism proposals. Hybrid options that include push and pull incentives have also become increasingly popular in recent years. Supporters and critics of these various incentive schemes have argued in favor of their relative merits and limitations, although the view that no mechanism is a perfect fit for all situations appears to be widely held. For this reason, the debate on the advantages and disadvantages of different approaches has been important for policy decisions, but is dispersed in a variety of sources. With this in mind, the aim of this paper is to contribute to the understanding of the economic determinants behind R&D investments for neglected diseases through the presentation of economic models of various incentive schemes. The analysis confirms that incentive schemes based on constant sums appear to be much less effective at inducing R&D investment by companies, and/or more expensive for the sponsor than simple linear incentive functions such as $F(C) = bC$. Finally, the work suggests that co-funded 'push' schemes with linear incentives $F(C) = bC$ are more effective than 'pull' schemes in inducing R&D effort by firms. Moreover, whenever intermediate goals are contractible, 'pay-as-you-go' incentives, that is when funding is provided sequentially upon reaching the agreed upon intermediate goals, could be even more preferable than 'push' schemes.

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C010_2012

Applications and Delivery Options for Antisense Therapy in Cellular Models of Inherited Metabolic Diseases

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The use of antisense genetic therapy for RNA mis-splicing diseases has gained increased attention as the splicing changes account for up to 15% of all mutations, and with massive parallel genomic sequencing of individual patients the number of splicing mutations will be increased. Although the number of patients who can be potentially treated is low for each inherited metabolic disease (IMD), it represents an excellent therapeutic option representing a type of personalized molecular medicine which is especially relevant for diseases for which there is to date no efficient treatment. In this work we summarize the splicing modulations explored to date, especially targeted to deep intronic changes and the potential use to reprogram the splicing process using antisense therapy against intronic and exonic new or cryptic splice sites. In addition, we present our recent data in the investigation of new transporter structures that are thought to provide effective in vivo delivery. We are working on an octa-guanidine dendrimer covalently linked to specific morpholinos and also a new approach using locked nucleic acids monomers (LNA) bound to carbosilane dendrimers. We have successfully recovered the splicing

process in *PMM2, MUT, PTPS, PCCA, PCCB* and *ALDH7A1* disease cellular models, suggesting that we are closer to applying the antisense therapy in animal models.

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C011_2012

The Benefits of Multidisciplinary Phenotyping: Experiences from the Dutch CHARGE Outpatient Clinic

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Purpose: Multidisciplinary expert clinics on rare disorders can be a powerful tool for patient-driven research or translational research, and can even lead to key concepts for fundamental research. We present the research outcomes from our national CHARGE clinic and show how, through international collaboration, these expert clinics can make a big difference to our knowledge on patients with rare disorders and to their care. **Methods:** The Dutch CHARGE clinic was started in 2005 and currently has 70 patients with proven CHARGE syndrome in follow-up. All patients are seen every 1–2 years by a pediatric endocrinologist, ENT specialist and clinical geneticist. Other specialists are involved, depending on the patient's problems and questions, for example, a psychiatrist, speech therapist, gynecologist, cardiologist, or occupational therapist. All clinical data are stored in a dedicated database and research questions are extracted from the experiences of the team members who include several PhD students. **Results:** Our approach has led to a series of clinical and translational papers on CHARGE syndrome. The main achievements so far have been an online locus-specific database that contains all the identified *CHD7* mutations, a classification system for *CHD7* missense variants, insights into the pathogenesis of anosmia and pubertal delay along with recommendations for the surveillance of pubertal development in CHARGE syndrome, a guideline for *CHD7* analysis in CHARGE and Kallmann syndromes, an extensive inventory of heart defects due to *CHD7* mutations, and a further exploration of the phenotypic and etiological overlap between 22q11 deletion syndrome and CHARGE syndrome. Recently, several other CHARGE clinics have been set up based on the Dutch model, including in the United Kingdom, United States, Denmark and Australia. These clinics have equally high standards of phenotyping and are facilitating international research collaborations; they have already resulted in a broad international study on immunodeficiency in CHARGE syndrome. **Conclusion:** Multidisciplinary expert clinics not only improve patient care by concentrating experience of the syndrome and its problems, but can also initiate and greatly facilitate clinical and translational research, thereby further advancing patient care through the development of evidence-based guidelines and recommendations.

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C012_2012

A Cell-Based Drug Discovery Platform to Identify Novel Therapies for Friedreich's Ataxia

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Friedreich's ataxia (FRDA) is a degenerative disease caused by deficiency of the protein frataxin. To date, neither regulation of the native frataxin gene *(FXN)* promoter nor the precise mechanism of frataxin gene silencing is well understood. Importantly, the mutation causing FRDA is an expansion of GAA·TTC triplets in the first intron of the *FXN* gene that does not alter the protein-coding sequence but rather affects RNA polymerase II-dependent transcriptional elongation. Therefore, gene reactivation could provide therapeutic benefit to FRDA patients and we thus aim at elucidating the regulatory mechanisms of frataxin gene expression. To enable high-throughput screening approaches, we developed reporter cell lines for simple detection of endogenous human *FXN* gene expression in its natural genomic context by exploiting a zinc-finger nuclease (ZFN)-mediated genome editing approach. In order to study frataxin gene expression in the disease-relevant cell types, we are also working towards reprogramming our reporter cell lines into the cell types that are mainly affected in FRDA. First results from a pilot RNAi screen using this system will be presented.

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C013_2012

Rapamycin-Mediated Glycogen Synthase Inhibition Can Relieve Polyglucosan Neurotoxicity in an Adult Polyglucosan Body Disease Neuronal Model

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Background and Purpose: Adult polyglucosan body disease (APBD) is a rare neurometabolic disorder caused by mutations in glycogen branching enzyme 1 (GBE1), which enables glycogen branching, thus preventing the formation of insoluble polyglucosan in cells. While loss of function of GBE1 can evoke lethal childhood disorders such as glycogen storage disease type 4 (GSD-IV), other mutations, such as Y329S still allow residual GBE1 activity

and cause the late onset, slowly progressing APBD. The link between the accumulation of polyglucosans and APBD pathogenesis is unknown and its treatment is so far symptomatic. We attempted to relieve the burden of polyglucosan accumulation in neurons using the autophagy inducer rapamycin. **Methods:** GBE1 expression in rat embryo cortical neurons was suppressed by transduction with lentiviruses whose RNA genome encodes for shRNA sequences against GBE1. GBE1, the autophagy marker LC3 and glycogen levels were assessed by immunoblotting and indirect immunofluorescence using confocal microscopy. GBE1 and glycogen synthase (GS) activities were assessed by ^{14}C-glucose incorporation into glycogen. Apoptosis was assessed by flow cytometry, and intracellular morphology by transmission electron microscopy. **Results:** GBE1 knocked down neurons were apoptotic and showed glycogen accumulation, similarly observed in APBD patient-derived cells. Induction of autophagy by rapamycin was able to clear glycogen aggregates and rescue the GBE1 knocked down neurons from apoptotic death. Nevertheless, autophagic maturation was dispensable for the corrective effects of rapamycin, apparently mediated by inhibition of GS activity. **Conclusions:** Our data show that while rapamycin is a classical inducer of autophagy, its actual mode of action in clearing glycogen in this APBD model is through GS inhibition. Our results suggest a strategy for treating APBD based on down-modulation of the ratio between glycogen synthesis and branching, which would reduce the levels of neurotoxic polyglucosan. This approach might also be applied to reversing the polyglucosan accumulation observed in other neurological disorders, such as Alzheimer's.

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C014_2012

Patient Advocacy Needs Greater Focus on Patient Needs

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Acromegaly is a rare pituitary adenoma that most patients have never heard of when they are diagnosed. Medical and pharmaceutical industries are spending a tremendous amount of their time and energies in researching new treatments and surgeries to deal with rare diseases such as acromegaly without working to provide 3-dimensional care for their patients. While this dedication to research and development is essential to the physical survival of patients, it does nothing to help with the emotional survival of the patient and the family unit. This is where the use of third party patient advocates provides that key third dimension of patient support. The goal of this presentation is to demonstrate the value of third party patient advocacy groups and practical demonstration of how such services are best employed by the medical professional. Frequently, patients are sent out of the doctor's office with a prescription, and maybe a well-meaning comment of optimism. There are no local support groups, few inspirational books, and seemingly no one to talk with about their fears. This is further complicated with restrictions related to HIPPA. Even if a medical professional wanted to team up patients and loved ones with more seasoned patients and loved ones, the process is slow and cumbersome. While pharmaceutical companies have not adequately addressed the emotional needs of patients, they have begun to recognize the importance of working with third party specialists who focus solely on patient care and advocacy. One of the greatest services a medical or pharmaceutical company can do to support their patients is to strengthen third party advocacy groups led by patients, former patients, and people who have traveled the patient path and are willing to be a community leader. Patients are eager to have their voices heard, when the audience is genuinely interested in what they are saying. When genuine partnerships of support are built with patient advocates, patient needs and desires are communicated straight from the patient. When patients receive a diagnosis they may have been anticipating for months, years, or even decades, they need support. While there is a sense of relief when diagnosed with a long-suffering condition, there is an emotional cascade to follow. How those emotions are supported will have a significant impact on how the patients live with their medical condition.

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C015_2012

Towards a Cone-Directed Comprehensive Therapeutic Strategy in Retinitis Pigmentosa

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In retinitis pigmentosa, the loss of light-adapted visual responses is the key event leading to blindness. We shall describe the potential strategies to protect or restore cone function, our clinical experience in high resolution imaging of these cells, and methods to assess reliably and enhance the impact of such innovative therapies in daily life. We demonstrated that cone cell function loss might result from the loss of expression of rod-derived cone viability factor (RdCVF) consecutive to the degeneration of rod photoreceptor cells directly affected by causative mutations. Administration of RdCVF, irrespective of the gene defect, induced in relevant animal models a strong preservation of cone cell function related to the maintenance of rod outer segments. Recently, work conducted by Botond Roska with our group showed that in advanced cases, cone cell bodies of dormant cones can be reactivated by vectorization of halorhodopsins, i.e. chloride-pumps activated by light, thus restoring cone function through adequate stimulation. These experiments bring strong emphasis on the assessment of the status of cone photoreceptors during the course of the disease. We have followed longitudinally over the past years a cohort of over 3,000 patients and studied the morphology and function of cone photoreceptors, using novel high resolution imaging technologies and functional testing such as adaptive optics and segmentation of spectral-domain optical coherence tomography images. We show that cone outer segment degeneration during the course of the disease can be documented and that patients suitable for clinical trials testing neuroprotec-

tion or optogenetics can be selected on the basis of the presence of cone cell bodies and, respectively, the shortening or lack of cone outer segments. A comprehensive personalized set of therapeutic strategies can be tailored on this basis. In order to evaluate and document functional outcomes, we have developed novel tools for assessing reproducibly visual impairment and restoration, as well as palliative aids and associated training protocols. These include the development of virtual simulators, the construction of versatile environments reproducing daily life situations (e.g. apartment, street, shop, obstacles, …) and the implementation of monitoring tools (captors, cameras, multiparametric modelling).

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C017_2012

Porphyrias

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Introduction and Aims: The porphyrias are metabolic disorders caused by defects in enzymes responsible for biosynthesis of heme. In hepatic porphyria, the acute attacks are typified by a triad of symptoms: abdominal pain, neurological and psychiatric disorders, and in severe cases it can come up to respiratory paralysis and coma. Porphyria cutanea tarda (PCT, chronic hepatic porphyria) occurs with skin lesions especially on sun-exposed areas. The renal involvement in acute hepatic porphyria is represented by hyponatremia, urinary retention, tubulointerstitial nephritis, hypertension and chronic kidney disease. Most of our patients had a pain similar to renal colic ache with propagation forward and down till the genitals, associated with pallor, nausea, vomiting, fever and acute retention of urine, with the emission of dark urine which sometimes turn dark red. **Methods:** Our case study consists of 50 patients: 31 with acute intermittent porphyria (AIP), 10 with hereditary coproporphyria (HCP), 6 with PCT and 3 with erythropoietic protoporphyria. **Results:** 7 patients with AIP and 3 with HCP are treated with hemin i.v. (Normosang®), a life-saving drug for them, at a dose of 3 mg/kg/day with resolution of clinical symptoms and resumption of normal daily activities. Maltodextrin (Polycose®) is also used to a normocaloric and hyperglycaemic diet therapy. **Conclusions:** At the present time, porphyria mortality has been reduced since it first reached 20–25% in the first 5 years after the first attack. Prevention is most important. Administration of drugs that can induce the disease should be avoided, as well as common treatment medications, tonics and herbal remedies. A high carbohydrate diet in addiction to maltodextrin should be prescribed. This therapy represents a ray of hope for the survival of patients with porphyria, providing them with a therapeutic element of high compliance, low-cost, without the collateral effects of drugs and indisputable clinical results but with the future possibility of increasing once more the percentage of carbohydrate in diet.

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C018_2012

Therapy-Induced Late Toxicity in Children with Rare Diseases: Long-Term Follow-Up Is Needed but Gets Disrupted in Adolescence – A Study in Pediatric Oncology from the Swiss Childhood Cancer Registry

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Background: As with patients of other rare diseases, intensive therapy may alter cellular structure and organ function in childhood cancer patients (~200 new cases/year in Switzerland). Even decades after diagnosis, 3 out of 5 former patients suffer from chemo- and radiotherapy-associated late effects including second cancers and late mortality. Therefore, lifelong follow-up is needed for the majority of patients. However, during adolescence many patients stop adhering to recommendations putting themselves at risk for undetected and advanced late effects. **Aims:** We thus aimed to determine (1) the proportion of former patients still attending follow-up care at the time of study in adulthood. In those no longer attending: (2) the age at last follow-up attended, (3) whether they had attended a follow-up program specially focused on the phase of transition from pediatric to adult care in adolescence, and (4) whether they had been informed about the importance of continuing follow-up. **Methods:** In 2010 and 2011, we sent a questionnaire to 449 former patients registered from the Swiss Childhood Cancer Registry, diagnosed in 1990–2006, >5 years after diagnosis and aged ≥18 years. The questionnaire included several questions on follow-up care. **Results:** By 2012, 204 former childhood cancer patients replied (response rate 45%; mean age at survey = 21 years; mean age at diagnosis = 8 years). Overall, 41% (n = 86) reported to attend follow-up care. This did not differ by initial cancer diagnosis. Among those no more attending, the median age at last attended follow-up was 18 years (minimum: 3, maximum: 27, interquartile range: 15–20 years). Only 7 (6%) had attended a special follow-up program during adolescence. Forty-five percent (n = 53) had been told about the importance of continued follow-up care. **Conclusion:** In adolescence, a relevant number of former childhood cancer patients get lost to follow-up. Research to discover underlying causes and setting up follow-up structures within the health care system is needed. This may also be the case in other clinical disciplines with life-threatening rare diseases and aggressive therapy.

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C021_2012

Corneal Cystinosis in the Cystinosin Knockout Mouse: Development of a Quantitative Model to Evaluate Novel Therapies

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Purpose: (1) To characterize the time course of corneal cystine crystal development in the cystinosin (*Ctns*$^{-/-}$) knockout mouse model of cystinosis. (2) To assess quantitatively the effects of topical cysteamine therapy using in vivo confocal microscopy (CM). **Methods:** (1) 2 *Ctns*$^{-/-}$ mice and 1 C57BI/6 mouse were examined using in vivo CM at each of the following time points: 2, 3, 5, 7, 10, 12 and 14 months of age. Animals were then sacrificed and cornea blocks evaluated for cell morphology using phalloidin and for lymphocyte infiltration using CD45 antibodies. Corneal cystine crystal content was measured by calculating the pixel intensity of the crystals divided by the stromal volume. (2) Next, the crystal volume index (CVI) of one eye receiving topical cysteamine eyedrops (0.55%) for 4 weeks was compared to the contralateral eye in 5 *Ctns*$^{-/-}$ mice. **Results:** (1) Corneal crystals were identified in *Ctns*$^{-/-}$ eyes beginning at 3 months of age, increasing in density from 7–12 months (when animals begin to succumb to the disease and corneas become scarred and neovascularized). *Ctns*$^{-/-}$ mice (7 months and older) demonstrated cell infiltrates that stain positively for CD45, which is associated with progressive keratocyte disruption. At 12 months of age, decreased cell density and endothelial distortion were also detected. (2) Eyes treated with cysteamine drops for 4 weeks beginning at 5 months of age showed significantly less crystal accumulation compared to control eyes ($p < 0.001$) with only a 15% increase in treated eyes (p = ns) compared to 173% increase ($p < 0.04$) for untreated eyes. **Conclusions:** (1) CM identified corneal crystals starting at 3 months in *Ctns*$^{-/-}$ eyes, which subsequently demonstrate findings that are consistent with observations in human cystinosis. (2) Topical cysteamine inhibits crystal volume progression in the *Ctns*$^{-/-}$ mouse, again analogous to clinical observations in cystinosis patients. Taken together, these data support the use of CVI and the *Ctns*$^{-/-}$ mouse model as a promising tool for novel therapeutic development.

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Genomic Disorders

D001_2012

Frameshift Mutations in Hyaline Fibromatosis Syndrome (HFS) Reveal the Significance of Personalized Treatment in Patients

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Hyaline fibromatosis syndrome (HFS) is a rare autosomal recessive disease, which is caused by mutations in capillary morphogenesis gene 2 *(CMG2)*. This gene encodes a type I transmembrane protein, the physiological function of which is not clearly understood while its role as the main anthrax toxin receptor in mammals is well established. Mutations were identified throughout the gene. Among them, exon 13, which is one of the exons encoding the cytosolic tail of the protein, has shown to be a hot spot for frameshift mutations. Here we focused on the 3 sequenced frameshift mutations in this exon due to single or double base insertions or deletions. The 3 frameshift mutations all led to a decrease in mRNA in patient cells presumably due to targeting to the NMD (nonsense-mediated mRNA degradation) pathway. We next analyzed the consequences of the mutation at the protein level in both transfected Hela cells and fibroblasts from patients. One base insertion led to a protein with a shorter cytosolic tail, which was properly targeted to the plasma membrane and we are analyzing the functional effects of the mutation. Two bases insertion or one base deletion in the same codon also led to a truncated cytosolic tail with only one amino acid difference in sequence between them. Interestingly however, these mutant proteins did not exit the ER, underwent polyubiquitination and were rapidly targeted to the ER-associated degradation pathway. Transplantation of this truncated tail to other transmembrane or cytosolic proteins also led to a similar degradation. While the native CMG2 tail is intrinsically unstructured and highly polar, the mutant tail was predicted to have a high helical content and to be significantly hydrophobic. Thus, ironically, changing the cytosolic tail from a sequence that should not fold to a sequence that has structure but cannot fold properly, renders it a substrate for ER quality control, the identity of which remains to be identified. Therefore, even if saving the mRNA by inhibiting NMD pathway in patients, the proteins with these mutations would still get recognized and degraded. And treating patients with proteasome inhibitor like Bortezomib, as shown in previous studies on point mutations mapping to exons encoding CMG2 ectodomain, would not help the accumulated proteins get out of ER to reach the plasma membrane in this case. The implications in these findings highlighted the importance of personalized treatment of HFS patients.

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D002_2012

Mitochondrial Dysfunction and Purkinje Cell Loss in the Human Spastic Ataxia ARSACS

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Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) (OMIM: 270550) is a childhood-onset neurological disease resulting from mutations in the *SACS* gene encoding sacsin, a massive 4,579 amino acid protein of unknown function. Originally identified as a founder disease in Quebec, ARSACS is now recognized worldwide. Prominent features include pyramidal spasticity, peripheral neuropathy and cerebellar ataxia, but the underlying pathology and pathophysiological mechanisms are unknown. We have now generated sacsin knockout mice that display age-dependent neurodegeneration of Purkinje cells and modified mitochondrial function. Mitochondrial dysfunction is a common pathophysiological feature of major neurodegenerative diseases including Huntington's, Parkinson's, and Alzheimer's. We show that sacsin localizes to mitochondria in neurons and non-neuronal cells and that it interacts and co-localizes with dynamin-related protein 1, which participates in mitochondrial fission. Disruption of sacsin function leads to an overly interconnected and functionally impaired mitochondrial network. Mitochondria accumulate in the soma and proximal dendrites of neurons and there are striking alterations in the organization of dendritic fields and the morphology of dendritic spines that precede neuronal cell death. Our data reveals mitochondrial dysfunction/mislocalization as the likely cellular basis for ARSACS.

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D003_2012

Lamin B1 Overexpression Is Associated with Increased Stiffness of Nuclei Isolated from Human Skin Fibroblasts of Patients with Autosomal Dominant Leukodystrophy

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Autosomal dominant leukodystrophy (ADLD) is a rare, progressive and fatal genetic disease affecting patients in their late 40s. Duplication of a region of chromosome 5 encompassing the gene encoding lamin B1 *(LMNB1)* protein has been causally linked to ADLD in 9 pedigrees of different ethnic origin. In such kindreds, the duplication of the gene leads to overexpression of the LMNB1 protein. LMNB1 is one of the major components of the nuclear lamina, a filamentous meshwork underneath the inner nuclear membrane. LMNB1 forms a stable structure in the nuclear lamina and, together with other lamins (e.g. lamin A/C), contributes to determine nuclear mechanical stability. Consistent evidence indicates that lamin A (LMNA) mutations or absence significantly alter nuclear shape and elasticity. Here, we evaluated whether LMNB1 upregulation linked to ADLD could also alter nuclear morphology and mechanical properties. To this purpose, we have established primary cultures of human skin fibroblasts from 3 ADLD patients with *LMNB1* duplication and 6 age-matched healthy volunteers. LMNB1 protein levels were significantly increased in fibroblasts from ADLD affected patients, while levels of the cognate LMNA and LMNC proteins were apparently unchanged. LMNB1 upregulation was associated with altered nuclear morphology as shown by the reduced circularity index and presence of nuclear blebs. The elasticity of isolated nuclei was probed by atomic force microscopy (AFM) using spherical polystyrene beads mounted on silicon tipples cantilevers TL1 as indenters. Force maps covering a 16-μm^2 area were acquired on each nucleus. AFM analysis on nuclei isolated from control fibroblasts revealed that nuclear elasticity varied with the proliferating status of the cells, but not with aging in vitro. Therefore, to avoid confounding effects due to cell proliferation, we performed AFM analysis on nuclei isolated from ADLD and control fibroblasts synchronized by serum deprivation for 24 h. Notably, nuclei from ADLD fibroblasts were on average significantly stiffer than control nuclei, suggesting that LMNB1 upregulation impacts on nuclear mechanical properties. We are currently dissecting the specific contribution of LMNB1 to nuclear stiffness using mouse embryonic fibroblasts from LMNB1-deficient and wild type mice or cells with controlled overexpression of LMNB1. Further studies are warranted to evaluate the impact of nuclear abnormalities on gene expression and pathogenic mechanisms.

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D004_2012

Dogs Provide Clinically and Genetically Relevant Models for Rare Human Disorders

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Inherited disorders are common in humans and dogs, and the clinical signs in dogs often closely mimic human diseases. For an increasing number of canine diseases the molecular cause is known and often mutations are reported in the orthologues of the corresponding human diseases. However, canine genetic studies have also potential to identify new candidate genes for human diseases. For example, we identified *SERPINH1* as a new osteogenesis imperfecta (OI) gene in Dachshunds, and a recent independent study showed that coding mutations of the *SERPINH1* gene are also responsible for the recessive forms of human OI. Besides being useful for revealing specific disease genes, canine genetic studies even have the potential to reveal entirely new pathways. An example is that of ectodermal dysplasia (ED) in the Chinese Crested dog, which is caused by a 7-bp duplication in the coding region of *FOXI3*. This study indicated for the first time an essential role for a forkhead box (FOX) class transcription factor in hair and tooth development. There are still many human ED patients without causative mutation and FOX class transcription factors and genes in the related pathway are good functional candidates. In a current study we aim to identify the causative mutation for recessive inherited craniomandibular osteopathy (CMO) in certain Terrier breeds. CMO is characterized by a non-neoplastic proliferation of bone on the ramus of the mandible and/or the tympanic bulla. The disease in various respects resembles Paget's disease and infantile cortical hyperostosis (Caffey's disease) of humans. We mapped CMO to a 1.8-Mb region on dog chromosome 5 by GWAS. The mapped region contains no obvious functional candidate gene and therefore offers a chance to identify a new candidate gene for canine and human CMO. The identification of canine disease genes establishes clinically important animal models and offers unique opportunities to test new therapies. Dog is a large animal and physiologically and clinically closer to human than rodent models. Canine disorders resemble better human condition, which is useful in preclinical trials of new treatments. Thus, testing the feasibility and efficacy of new therapies in these genetically characterized large animal models may facilitate translation of curative treatments for rare human genetic diseases.

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D005_2012

Mutations in the *PLEKHG5* Gene Cause Peripheral Neuropathy

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Inherited peripheral neuropathy (also called Charcot-Marie-Tooth disease) includes a clinically and genetically heterogeneous group of neuromuscular disorders characterized by progressive distal muscle weakness and atrophy, foot deformities, and distal sensory loss. Using a homozygosity mapping approach in a consanguineous Moroccan family with a late-onset, recessive, and predominantly demyelinating form of hereditary neuropathy, we identified a 7-bp homozygous duplication (c.1143_1149dupl7 – TGAAGAC) in the *PLEKHG5* gene on chromosome 1p36 in all affected members of this pedigree. This DNA change introduces a premature stop (E384X) in the reading frame and is predicted to result in a null allele. Our expression analysis of the mouse *Plekhg5* gene in developing and adult central and peripheral neural systems revealed its temporally regulated endoneurial expression, indicating a role in Schwann cell myelination. Interestingly, it was previously reported that a homozygous missense change (c.1940T>C) in *PLEKHG5* leads to an autosomal recessive form of lower motor neuron disease with childhood onset. Together with our results, these data indicate that different mutations in *PLEKHG5* lead to clinically diverse outcomes, perhaps related to a reduced activity versus the complete absence of the protein. Regardless of the molecular mechanisms of action, intact *PLEKHG5* function seems to be necessary for normal function of both peripheral neural system glia and neurons.

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D006_2012

Development of Pathophysiologically Based Biochemical Assays to Identify Small Molecular Weight Compounds for the Treatment of Myotonic Dystophy Type I

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Myotonic dystrophy type I (DM1) is a disabling, genetic disease affecting multiple organ systems, including skeletal and cardiac muscle, central nervous system, gastrointestinal tract, endocrine glands and eye, with no causal treatment available. This disease is caused by expanded CTG triplet repeats in the 3′ UTR of the myotonic dystrophy protein kinase *(DMPK)* gene. Disease severity is correlated to the repeat expansion size: Normal subjects

harbor less than 37 CTG repeats, whereas in subjects with congenital forms the repeat length can exceed 2000 triplets. On the RNA level such expanded CUG repeats (CUGexp) form hairpin structures, which lead to ribonuclear inclusions. More specifically, the RNA with expanded CUG repeats sequesters the splice-factor muscleblind-like 1 (MBNL1), which is involved in alternative splicing. Lack of available MBNL1 leads to mis-regulated alternative splicing of many different genes, explaining thus the multisystemic defects in DM1. We wish to identify small molecular weight compounds that liberate sequestered MBNL1 from CUGexp-RNAs in affected organs. In order to identify such small molecular weight compounds for the treatment of DM1, we developed different pathophysiologically based biochemical assays. Beside an improved gel-shift assay, and ELISA-based assay, we also developed an assay based on differential scanning fluorometry. Each of the different assays has its specific advantages as well as disadvantages. These assays could be applied to drug screenings for other rare RNA-mediated diseases like myotonic dystrophy type II, fragile-X tremor ataxia syndrome and different types of spinocerebellar ataxias.

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D007_2012

Analysis of Superoxide Dismutase Gene Family in Patients with Autistic Spectrum Disorder in the Slovenian Population

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Autistic spectrum disorder (ASD) is a group of neurodevelopmental disorders with common impairments in social interaction, language development and communication, with rather unknown etiology. ASD has an incidence of ~4/1000, with 3:1 male to female ratio. The prevalence of ASD for siblings of ASD patients is much higher (2–6%), indicating significant genetic background of the syndrome. Several studies indicate that levels of oxidative stress markers are significantly higher in ASD patients compared to healthy individuals. Superoxide dismutases (SOD1, SOD2 and SOD3) catalyze superoxide radicals into oxygen and hydrogen peroxide. Thus they are important in regulating homeostasis of reactive oxygen species (ROS) and reduce levels of oxidative stress. The aim of this study was to analyze 5′ UTR and coding regions of genes of the superoxide dismutase *(SOD)* family in 96 individuals diagnosed with ASD and compare the results with those from healthy unrelated controls to establish correlation between genetic variation of *SOD* genes and susceptibility for ASD. In our group, 70 individuals were diagnosed with classical autism, while 26 were diagnosed with Asperger syndrome. All individuals or their parents signed informed consent and volunteered for participation in this study. Whole blood samples were taken from each individual and DNA isolation was performed according to established laboratory protocols. We screened for genetic variations of *SOD* gene family by high resolution melting (HRM) analysis. All detected genetic variants were confirmed by DNA sequencing. Results were statistically evaluated by comparison to healthy control subjects from the Slovenian population. We identified 56 different genetic variants. None of the genetic variants analyzed so far had any statistically significant correlation with ASD or any specific ASD subgroup. Therefore, these preliminary results suggest that *SOD* gene family variations might not be correlated with ASD etiology.

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D009_2012

Cystic Fibrosis Physiotherapy Treatment in Daily Routine of Newborn Babies and Toddlers

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Purpose: This study is aiming to enhance the quality of life in cystic fibrosis (CF) children, integrating, with the help of parents, the treatment in daily routine and promoting the joy of movement in a safety environment. **Methods:** This study was conducted in the Romanian National Cystic Fibrosis Centre in 2010–2011, including 20 patients aged between 6 months and 3 years. The initial and final assessment was made with the help of parents who chose the 6 most important items for them from quality of life scale in CF: quality of sleep, fatigue during play activities, nutritional status, coughing, clinical status, number of hospitalizations. The chosen therapeutic techniques were: modified postural drainage, contact breathing, vibrations, therapeutic and facilitation postures, massage, games for blowing, incentive games, and stretching and strengthening exercises. **Results:** We noticed improvements regarding all items in the study group: deep sleep quality increased with 20% and this had a positive effect on weight gain (increasing on average by 30% from varying degrees of growth bankruptcy to normal weight). Fatigue during play activities decreased due to secretion mobilization, infection reduction, improvement of sleep quality and muscle strength growth. The number of hospitalizations also decreased from 60% to 40%. **Conclusions:** Combining airway clearance techniques and physical activities could optimize quality of life in toddlers with CF. Physiotherapy must be included in the daily program of any patient with CF and must be conducted by parents not only by physiotherapists.

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D010_2012

Mirror Phenotypes Associated with 16p11.2 Rearrangements

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Recent extensive GWAS identified numerous loci associated with obesity, but they only account for a small fraction of the heritability of this trait. We previously reported a highly penetrant form of obesity resulting from a heterozygous deletion of 600 kilobases at the 16p11.2 locus. Deletions identified from GWAS data in 16,053 individuals from 8 European cohorts were absent from healthy non-obese controls and accounted for 0.7% of our morbidly obese cases (body mass index, BMI $\geq$40 kg/m^2, p = 6.4 $\times$ 10^{-8}, OR = 43.0). In contrast to obesity, few genetic variants underlying clinical conditions associated with being underweight (BMI <18.5) have been reported. We identified 138 carriers of the reciprocal duplication from individuals clinically referred for developmental or intellectual disabilities (DD/ID) or psychiatric disorders, or recruited from population-based cohorts. These carriers show significantly reduced postnatal weight and BMI. Half of the boys under 5 years of age are underweight with a probable diagnosis of failure to thrive; whereas adult duplication carriers have an 8.3-fold increased risk of being clinically underweight. Gene dosage correlates with changes in transcript levels for genes mapping within the duplication, but not in flanking regions. The reciprocal impact of these 16p11.2 copy number variants indicates that severe obesity and being underweight could have mirror etiologies, possibly through contrasting effects on energy balance. We are conducting a systematic characterization of associated clinical symptoms, focusing on mirror eating behaviors and psychiatric disorders, including autism and psychotic symptoms. Structural and functional imaging is also being performed. Progress in these studies will be reported. This study highlights a promising strategy for identifying missing heritability in complex traits.

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D011_2012

Noonan-Like Syndrome with Loose Anagen Hair Is Caused by Different Mutations in *SHOC2*

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Noonan-like syndrome with loose anagen hair (NS/LAH; MIM #607721) is a clinically distinct entity in the spectrum of neuro-cardio-facial-cutaneous disorders, mainly characterized by typical facial features, growth deficiency (frequently with growth hormone deficiency), easy pluckable, sparse, slow-growing hair, darkly pigmented skin and developmental delay. In 2009, Cordeddu et al. identified a germline mutation in *SHOC2* (c.4A>G, p.Ser2Gly) present in all patients published so far. Because of its particular functional mechanism (creation of a recognition site for N-terminal myristoylation), this has been supposed to be the only causative *SHOC2* mutation. We report on a 4.5-year-old girl, born to healthy, non-consanguineous German parents after an uneventful pregnancy at 37+3 weeks of gestation with normal birth measurements [3300 g weight (75th percentile), 50 cm length (50th percentile) and 33 cm OFC (25th percentile)]. Her psychomotor development was mildly delayed. Hand X-rays at age 15 and 25 months showed a significantly delayed bone age of 6 and 9 months, respectively. At age 2 7/12 years, she was first presented because of short stature with relative macrocephaly, delayed closure of large fontanel, short, sparse, brittle blond hair and a grayish complexion. Measurements were 82.3 cm length (3 cm <3rd percentile), 11.1 kg weight (10th percentile) and 49 cm OFC (50th percentile). Endocrinological evaluation revealed a neuroendocrine dysfunction and growth hormone therapy was started at age 3 8/12 years. Re-evaluation at age 4 7/12 years showed a length of 97.7 cm (almost 3rd percentile), a weight of 15.8 kg (25th–50th percentile), and an OFC of 52 cm (90th percentile). Echocardiography, hair microscopy, copper and coeruloplasmin levels, array analysis and c7orf11 sequencing were all normal. The DYSCERNE experts suggested *SHOC2* mutation analysis. The typical mutation c.4A>G was excluded, but a novel previously undescribed mutation in exon 2 (c.517A>G, p.M173V) of initially unknown significance was identified and confirmed in a second tissue (saliva sample). Analysis of both parents indicated de novo occurrence. The de novo occurrence of this *SHOC2* mutation combined with the typical clinical phenotype suggested the mutation to be pathogenic, thus being the first mutation different from the single mutation described in all earlier published patients. Therefore, extended sequence analysis should be performed in absence of the c.4A>G mutation in patients with typical *SHOC2* phenotype.

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D012_2012

Disorders of Sexual Development: Identifying New Genes and Pathways Involved in the Sexual Determination of the Human Gonad

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Disorders of sexual development (DSD) are rare pathologies affecting 1 baby in every 3000 births. In such pathologies, the distribution of sex chromosomes (chromosomal sex), the differentiation of the embryonic gonads (gonadal sex) or the action of sexual hormones (hormonal sex) can be affected. In the case of gonadal dysgenesis (GD), where the determination of the gonadal sex is altered, the genetic etiology remains unknown for almost half of the cases, even though several causative genes, such as *SOX9*, *FOXL2*, *SF1*, *DAX1* or *WNT4*, have been identified in past genetic studies. The recent burst in the development of high-throughput methods for the genetic testing of individuals now gives us new tools to investigate unresolved cases of DSD with the final goal to identify new factors involved in the sexual development of the human gonad. For this purpose, we gathered a large cohort of 46,XX and 46,XY GD and searched for copy number variations (CNVs) by CGH arrays and single nucleotide variants (SNVs) using deep exome sequencing. Patients who have a normal karyotype, complete clinical data and unknown genetic etiology for their pathology were selected for analysis. The genomic DNA from 38 cases of 46,XY DSD and 18 cases of 46,XX DSD have been collected and are currently analyzed by array CGH and deep exome sequencing. Up to now, we identified and validated 2 causative CNVs in 46,XY GD patients, including a duplication of the WNT-pathway regulator *DAAM2* as well as a deletion of *HMGCS2*, an important gene for sexual dimorphism in mouse. Parallel to that, 1 putative causative SNV has been identified in a case of 46,XX GD and remains to be validated. Globally, our strategy appears efficient in bringing new perspectives in the genetic analysis of GD. This work is currently ongoing, and by proposing an optimized protocol for the clinical characterization of each case and by prioritizing the analysis of complete families, we should be able to provide new insights into the genetics of human sexual determination.

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Hyaline Fibromatosis Syndrome Inducing Mutations in the Ectodomain of Anthrax Toxin Receptor 2 Can Be Rescued by Proteasome Inhibitors

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Hyaline fibromatosis syndrome (HFS) is a human genetic disease caused by mutations in the anthrax toxin receptor 2 gene *(ANTXR2* or *CMG2)*, which encodes a membrane protein thought to be involved in the homeostasis of the extracellular matrix. Little is known about the structure and function of the protein and the genotype-phenotype relationship of the disease. Through the analysis of 4 patients, we identify 3 novel mutants and determine their effects at the cellular level. Altogether, we show that missense mutations that map to the extracellular von Willebrand domain or the here characterized Ig-like domain lead to folding defects and thereby to retention of the mutated protein in the endoplasmic reticulum. Mutations in the Ig-like domain prevent proper disulfide bond formation and are more efficiently targeted to ER-associated degradation. Finally we show that CMG2 can be rescued in fibroblasts of some patients by treatment with proteasome inhibitors and that CMG2 is then properly transported to the plasma membrane and is functional, identifying the ER folding and degradation pathway components as promising drug targets for HFS.

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Host- and Pathogen-Derived Factors in Chronic Mucocutaneous Candidiasis

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Chronic mucocutaneous candidiasis (CMC) is a rare but severe disease characterized by persistent or recurrent infection of the nails, skin or oral mucosa caused by fungi of the genus *Candida*, mainly *Candida albicans*. The lesions are clinically heterogeneous and respond poorly to anti-fungal treatment. Only lately the first genetic etiologies of this rare disease have been described, including an autosomal dominant (AD) mutation in the gene encoding IL-17F, resulting in a deficiency of this cytokine, and an autosomal recessive (AR) mutation in the gene encoding the IL-17 receptor A (IL-17RA), resulting in a deficiency of the receptor for IL-17 cytokines. However, additional parameters may predispose for disease. In particular, the pathogenicity traits of the fungus may impact greatly on disease progression versus resolution. *C. albicans* is an opportunistic pathogen that lives as commensal in healthy individuals and only causes disease when host defenses are breached. It is believed that the host immune system alone determines the balance between commensalism and pathogenicity. However, the genetic traits of different clinical isolates may have a strong impact on their pathogenicity and on the consequence for the host, namely colonization versus invasion and disease. A detailed understanding of the molecular and cellular mechanisms that lead to the development of disease symptoms, combined to detailed knowledge of antifungal immune mechanisms, is indispensable for an integrated understanding of the disease. This project will use an integrated approach to evaluate the relative importance of host- and pathogen-derived factors in the development of a rare infectious disease. Applying an original, innovative and interdisciplinary approach that shall combine in vivo and in vitro studies using immunologic, genomic and proteomic techniques, we propose to identify new host- and pathogen-derived factors that contribute to the development and control of CMC. This project shall help to better understand the molecular pathogenesis of CMC with the aim to identify novel therapeutic targets and diagnostic markers, and to promote the development of novel antifungal strategies.

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A Muscular Dystrophy-Causing Lamin Mutation Interferes with Differentiation-Specific Relocation of a Muscle-Specific Promoter

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Chromatin is non-randomly organized in the interphase nucleus with heterochromatin often being found adjacent to the nuclear lamina. To see if gene position facilitates heritable repression states during cell differentiation, we have visualized developmentally controlled promoters in *C. elegans*. The position of low-copy integrated transgenes containing developmentally regulated promoters *(myo-3::mCherry* or *pha-4::mCherry-H2B)* and arrays of LacO sites were examined during development. In early embryos, inactive tissue-specific promoters were randomly distributed throughout the nuclear volume. During cellular differentiation, transgene position depended strictly on transcriptional status. In 4 different tissues repressed transgenes were shunted to the nuclear periphery, while active promoters were sequestered internally. In contrast, large heterochromatic arrays were sequestered at the nuclear envelope even in embryonic nuclei. To examine the role of lamin in heterochromatin anchoring, we performed RNAi of the unique worm lamin gene, and tested a point-mutated lamin that causes Emery Dreifuss muscular dystrophy (EDMD) in humans. Loss of lamin derepresses and delocalizes heterochromatic arrays, whereas the EDMD point mutation interfered with the release of active muscle-specific promoter arrays, uniquely in muscle cells. The mutation reduces expression from the array-borne *myo-3* promoter and leads to morphological disruption of actin fibers and sarcomeres. The worms lose muscular coordination as in human EDMD. We have thus reconstituted a human disease in *C. elegans* and find that a point-mutated lamin can lead to tissue-specific defects in chromatin position and tissue structure. The effects of the disease-causing mutation in lamin are unlike the effects of lamin deletion, and therefore show that this EDMD-linked mutation acts in a dominant fashion to provoke muscle-specific defects in nuclear organization and tissue function.

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High-Throughput Sequencing of the 22q11.2 Deletion in Velocardiofacial Syndrome to Study Significant Variants for Schizophrenia

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Velocardiofacial syndrome (VCFS) is a microdeletion in 22q11.2 ranging between 1.5 and 3 Mb, containing about 60 genes, with an incidence of 1 in 4,000 live births. One third of VCFS patients develop schizophrenia during early adulthood. The incidence of schizophrenia in patients with VCFS is 30 times higher than in the general population, thus making VCFS an important risk factor for schizophrenia and suggesting a strong correlation between it and the 22q11.2 microdeletion. Based on the high incidence of schizophrenia in these individuals, we hypothesized that some patients with VCFS develop schizophrenia due to hemizygosity of one or more critical regions in the 22q11.2 portion of the genome. Paired-end libraries were prepared from 38 VCFS patients, half with schizophrenia or schizo-affective disorder, and half without. Agilent's target enrichment capture was performed for a 3-Mb region spanning the 22q11.2 area. The enriched regions from the healthy chromosomes were then sequenced on an Illumina GAIIx. The reads were then aligned and mapped, obtaining at least 8× coverage in 98% of the region. On average, 16 non-synonymous variants were found per sample. After preliminary analysis a trend was identified among patients with a clear and confirmed schizophrenic phenotype (8 out of 16). Fisher association test reports a signal of at least 1 intergenic SNP ($p < 0.05$ in 8 out of 16 affected and none of the 16 non-affected). Therefore, a larger cohort of patients with a clear schizophrenic phenotype is needed to confirm the findings of this preliminary study.

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Sedlin and Prostaglandin E2 Dehydrogenase – Interactions and Implications for Spondyloepiphyseal Dysplasia Tarda

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Spondyloepiphyseal dysplasia tarda (SEDT) is a rare X-linked, late-onset skeletal disease. Affected individuals develop phenotypes in their early childhood, displaying barrel-shaped chests, vertebral body malformation, flattened disc spaces and premature osteoarthritis in weight-bearing joints. The disease was found linked to the gene *SEDL (TRAPPC2)* coding for the protein sedlin. Sedlin is one of the subunits of the TRAPP (transport protein particle) complex, which is responsible for vesicle tethering during endoplasmic reticulum-to-golgi transport. Although sedlin is known to function in intracellular trafficking, the reason why mutations in a trafficking protein lead to a skeletal disease remains unknown. To address this, 4 missense mutations (D47Y, S73L, F83S and V130D) of sedlin observed in SEDT patients were studied. Except D47Y, the other 3 mutations cause proteosomal degradation of sedlin in cultured cells, whereas the D47Y mutation had a minor effect on Bet3 binding to sedlin. Pull-down assay was performed to identify novel sedlin interacting partners. 15-Hydroxyprostaglandin dehydrogenase (PGDH) was pulled down and the interaction was confirmed in cell culture system. Sedlin activates PGDH activity in vitro. By confocal microscopy, sedlin was also found to colocalize with PGDH in the cytosol. PGDH catalyzes the degradation of prostaglandin E2, which affects cartilage and bone growth. Further investigation is ongoing to understand the function of sedlin and the mechanism of disease for SEDT.

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