Rare 2020

The 3rd International Congress on Rare Diseases

12-13 March 2020 | Berlin, Germany

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Abstract Book

Poster Board No. 50

General Challenges in Rare Disease

Clonal Transformation in Patients with Aplastic Anemia Associated with Paroxysmal Nocturnal Hemoglobinuria (AA/PNH) After Successful Immunosuppressive Therapy (IST)

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AA patients with long-term survival after immunosuppressive therapy have a risk of transformation to MDS or AML. AA is often associated with PNH (AA/PNH) also able to clonal transformation. We have analyzed the data of 25 patients with confirmed AA/PNH, initially without the bone marrow clonal cytogenetic abnormalities, which were observed for ≥3 years after IST. The diagnosis of SAA was established in 19 patients,NSAA – in 6. Of them 23 patients received combined IST (ATG + cyclosporine) and 2 patients with NSAA – only cyclosporine. Complete remission was achieved in 18 (72%) patients, of them in 2 (males, 42 and 40 years) with SAA (8% of the overall group) experienced a transformation to MDS/MPN (CMML) (patient 1) and MDS-EB1 (patient 2). On the moment of the transformation, the period from AA diagnosis was 158 and 115 months in the 1st and 2nd case, respectively, from the statement of remission – 117 and 69 months. Patients received combined IST and had long periods of achieving stable complete remission (41 and 46 months). At baseline patient 1 had minor PNH clone (0.1 -1.3%) and patient 2 – 15-17.9% PNH cells. In remission the gradual increase of the clone to 34.9% for 4 year-period was observed in patient 2. When stating clonal transformation, characteristic for both patients was the complete disappearance of the PNH clone. Thus, regardless of the PNH clone size, the patterns of clonal transformation in AA/PNH patients are similar and such patients need a long-term follow-up.

Poster Board No. 51

General Challenges in Rare Disease

Hickam`s Dictum Versus Occam`s Razor: Coexistence of Rare Disorders

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Background: Multisystemic involvement, parental consanguinity and family history rise clinical suspicion of rare disorders. They may not have disease-specific symptomatology. Occam`s razor means patient's symptomatology must be explained with single simplest explanation. Whereas, Hickam`s dictum states patient`s clinical findings may be secondary to two or more pathologies.

Objective: We aim to highlight diagnostic odyssey of rare disorders.

Method: Retrospectively, medical records of patients with molecularly-confirmed multiple rare diseases were reviewed.

Results: Patient-1 is a 12-year-old female who had parental consanguinity, hepatomegaly, hypoglycemia, myopathy, stomachache, and arthritis. She was diagnosed as having glycogen storage disease (GSD) type-3 and familial Mediterranean fever.

Patient-2 was a 4-month-old female with parental consanguinity and deceased siblings with biotinidase deficiency. Despite treatment, she had hypotonia. Congenital myasthenic syndrome type-5 was detected via whole exome sequencing (WES).

Patient-3 and 4 are brothers who have GSD-3 presenting with hepatomegaly, hypoglycemia, and myopathy. Both had unexplained epistaxis, prolonged partial thromboplastin time and low Factor-IX (1-5%) levels. Diagnosis was Hemophilia-B.

Patient-5 is a 2-year-old female who had parental consanguinity, psychomotor retardation, microcephaly, and normal anion gap metabolic acidosis. WES detected carbonic anhydrase deficiency type-II and Galloway-Mowat Syndrome.

Patient-6 is a 27-month-old male who showed hepatomegaly, hypoglycemia, coarse facies, and Mongolian spot. The diagnoses were GSD-Ia and mucopolysaccharidosis-IIIb.

Patient-7 is a 9-year-old male who was diagnosed with biotinidase deficiency through newborn screening program. Anemia persisted despite iron supplementation, and diagnosis was glucose-6-phosphate dehydrogenase deficiency.

Conclusion: Collaborative effort is needed for diagnosis of patients with multiple rare diseases. Misdiagnosis, delayed diagnosis, unnecessary tests and incorrect treatments are frequent. Open-mindedness for possibility of multiple diagnoses and complex diagnostic methods such as WES are helpful.

Poster Board No. 52

General Challenges in Rare Disease

Some Hypocoagulation Changes in The Hemostasis System in Patients with Paroxysmal Nocturnal Hemoglobinuria (PNH) During Eculizumab Therapy

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PNH is rare acquired clonal blood disease characterized by chronic intravascular hemolysis and thrombophilic state. Thrombosis is the leading cause of death for PNH patients. Thrombosis in patients with PNH is multifactorial and respectively routine coagulogram is usually not very informative and traditional anticoagulant therapy is ineffective. Effective prevention and therapy for thrombotic complications is eculizumab due to the inhibition of complement.

The blood clotting system was examined in 5 patients with different PNH clone size (from 48% to 98%) receiving eculizumab therapy. In addition to the usual indicators, the activity of factor VIII, antithrombin, protein C and S, D-dimer level and platelet aggregation function were determined before and after therapy. Initially, all 5 patients showed signs of hypercoagulation (significant increase in activity of factor VIII, increase of D-dimer). During the treatment of eculizumab, our data were indicating a tendency to decrease in activity of factor VIII and D-dimer levels in 3 out of 5 patients. It should be noted that the indicators of the anticoagulant system (antithrombin, protein C and S activity) did not change and were within normal values throughout the study in all patients with therapy. Aggregation function in 2 out of 5 patients examined decreased in the study with collagen and ADF.

Thus, some patients receiving eculizumab treatment showed a tendency to reduce individual factors of hypercoagulation state of the hemostasis system, which may indicate some anticoagulant and antiaggregant effects of eculizumab. These results require further research.

Poster Board No. 53

Miscellaneous Rare Diseases

Mitochondrial Complex IV Deficiency in A Child with Growth Retardation and Cerebellar Ataxia

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Background: Mitochondrial complex-IV is a multi-subunit protein encoded both by nuclear and mitochondrial DNA. Autosomal recessively inherited *COX20* gene mutations cause complex-IV deficiency which are associated with a unique phenotype that includes early-onset hypotonia, ataxia, areflexia, dystonia, dysarthria, and sensory neuropathy. To date, only seven patients were reported, three patients were of Turkish origin.

Case Presentation: An 8-year-old Turkish boy born at term with normal weight to consanguineous Turkish parents was admitted to hospital with growth retardation. His developmental milestones were appropriate for his age until 18 months of age. Then, he developed a progressive wide-based gait and frequent falls. On admission, his weight was 22 kg (-2,85 SDS), height was 125 cm (-2,71 SDS). Significant dysmetria and dysdiadochokinesia were observed. He had dystonic posturing of both hands during his attempts to walk. There were no areflexia, and sensory neuropathy. Cardiac, ophthalmological, and audiological evaluations were completely normal. MRI showed cerebellar atrophy. All metabolic investigations revealed normal results. Genetic testing for spinocerebellar ataxia panel was negative. Whole exome sequencing detected a homozygous novel c.190AC mutation in *COX20* gene. This variant was predicted to be disease-causing by *in silico* predictive tools. His parents were heterozygous for the same mutation.

Conclusion: Mitochondrial diseases are complicated and difficult to diagnose as they have bimodal pattern of inheritance. Also, it is important if these ultra-rare diseases share a common phenotype or not. As limited cases published in the literature, we report this interesting case with a novel mutation in order to highlight the phenotypic spectrum.

Poster Board No. 54

Miscellaneous Rare Diseases

Rare Disease in Organ Donors. 24-Mounthes Experience in Italy

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Introduction: Rare diseases (RD) are an eterogeneus group of patologies that are not trasmitted from organ donor to recipients. Nevertheless organs from these donors can presents functional deficit that could affect patient and/or graft survival. Aim of this work is to analize the incidence of rare disease in our organ donor population to support clinicians in the decision to use these organs.

Materials and Methods: We retrospectively assessed the incidence of RD in organ donors reported from July 2017 to June 2018, the risk attributed, the transplanted organs and the follow-up of the recipients.

Results: In 24 months, we had 19 donors (1%) affected by a RD (16 with a certain diagnosis, 3 with a suspected diagnosis). In 4 cases the donor hesitated in opposition, in 4 the risk attributed was unacceptable, in 1 standard, in 5 acceptable, in 2 negligible, in 3 a different risk was attributed depending on the organ considered. 4 donors were rejected by transplant centers, 8 were accepted with 18 transplanted organs (2 heart, 3 livers and 13 kidneys) in 17 patients. 3 recipients died for causes not related to MR, after a median follow-up of 9 mounthes (range 4-18) 14 are alive with a functioning organ.

Conclusions: The evaluation is affected by the short times of the donation process which do not allow genetic or histological insights for each organ. Furthermore, the heterogeneity of RD and the unavailability of literature require a particular case-by-case evaluation. Therefore, a group of expert geneticists and transplant clinicians has been set up at the Superior Health Council to support surgeons in assessing the suitability of the organ, ensuring compliance with the relative safety and quality of the transplant.

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Poster Board No. 55

New Treatment Modalities

Acetyl-Leucine Slows Disease Progression in Lysosomal Storage Disorders

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Introduction: Acetyl-DL-leucine (ADLL) is a derivative of the branched amino acid leucine. In observational clinical studies ADLL improved symptoms of ataxia in patients with the lysosomal storage disorder (LSD) Niemann-Pick disease type C (NPC).[1, 2] We investigated ADLL and its enantiomers acetyl-D-leucine (ADL) and acetyl-L-leucine (ALL) in *Npc1*$^{-/-}$ mice.

Methods: Affected mice (Npc1-/-, Hexb-/-) and controls (Npc1+/+, Hexb+/+) were included. Behavioral tests were performed (gait analysis, motor function assessment, incl. strength and coordination). Biochemical analyses (Western blot, ADP/ATP and NAD/NADH, sphingoid base, glycosphingolipid, cholesterol) were performed. Moreover, lysotracker green and propidium iodide staining, filipin staining as well as immunohistochemistry were conducted. Clinical observational studies of 13 adult NPC (12 on miglustat) and 3 GM2-gangliosidoses patients treated with ADLL were included.

Results: ADLL, ADL and ALL in symptomatic *Npc1*$^{-/-}$ mice all improved ataxia. When ADLL and ALL were administered pre-symptomatically to *Npc1*$^{-/-}$ mice, they both delayed disease progression and resulted in a modest extension to life span, whereas ADL did not. These data are consistent with ALL being the active neuroprotective enantiomer. Altered energy metabolism was implicated as a potential mechanism of action of the active L enantiomer in *Npc1*$^{-/-}$ mice. When miglustat and ADLL were used in combination significant synergy resulted. Disease progression rates were slowed after 12 months of treatment. A neuroprotective effect of ADLL was also observed in a mouse model, and clinical benefit observed in GM2 gangliosidosis` patients in observational clinical studies.

Conclusions: Taken together, we have identified an unanticipated neuroprotective effect of ALL, supporting its further evaluation in clinical trials in LSD.

Poster Board No. 56

New Treatment Modalities

N-Acetyl-L-Leucine for Niemann-Pick Disease, Type C, GM2-Gangliosidosis and Ataxia Telangiectasia: Three Multinational, Multicenter, Open-Label, Rater-Blinded Phase II Clinical Trials

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Introduction: N-Acetyl-L-Leucine (IB1001/ALL) clinical trials are three, phase II studies which investigate ALL for three rare, autosomal-recessive, neurodegenerative disorders: Niemann-Pick type C (NPC), GM2-Gangliosidosis (GM2) and Ataxia-telangiectasia (A-T). The mutual aspects of their clinical presentation, and mechanism of action of ALL, enabled a single master protocol to be developed, implementing both an innovative trial design and novel primary endpoint, better suited to these small, inhomogeneous patient populations.

Methods: The IB1001 studies investigate the symptomatic and disease-modifying effects of ALL. Screening of patients ≥6 years (Europe) AND ≥18 years (USA) occurs at 12 centers across Germany, Spain, Slovakia, UK, and USA. Patients who have completed the Parent Study (Fig.1 and 2) may be included into an Extension Phase (Fig. 3) The dosage varies from 2 to 4 g/day, based on patients' age/weight. A novel primary endpoint, the Clinical Impression of Change in Severity (CI-CS), was developed, based on two independent, blinded raters comparison of videos of the patient's change in performance from baseline to the end of treatment, and the end of treatment to the end of the washout on either the 8 Meter Walk Test (8MWT) or the 9 Hole Peg Test, Dominant Hand (9HPT-D).

Results: Recruitment is ongoing for all three studies. Approximately 39 patients per study will be screened. As of 15 January 2020, 29 NPC, 13 GM2, and 1 A-T patients have been enrolled.

Conclusions: Due to the clinical heterogeneity of these orphan populations, a novel primary endpoint is implemented to better demonstrate the clinically meaningful effect of ALL treatment.

Poster Board No. 57

New Treatment Modalities

New Approach for Treating Hemophiliacs with Inhibitors Using Fc-Proteins and NK Cells

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Background: Fc fusion proteins are used in replacement therapy of genetic deficiency diseases to prolong half-life.

Objective: To determine if Fc-FVIII (used in the treatment of Hemophilia A) could interact with FcgR.

Methods and Results: Immune system cells bear Fc receptors. Fc-mediated interactions with these receptors, can lead to the activation or inhibition of function. We studied a panel of FDA-approved Fc-fusion proteins in terms of binding and activation of Fc-receptors. Using human Fc gamma receptor (FcγR) reporter cell lines, we demonstrated that Fc-FVIII bound and stimulated reporter cell lines expressing activating and inhibitory FcγRs (I, IIA, IIB, IIIA).

The finding that Fc-FVIII bound and activated cells via FcγRIIIA was studied in more detail. FcγRIIIA is expressed on most natural killer (NK) cells. We demonstrated that Fc-FVIIII activated NK cells as measured by IFNγ, perforin and granzyme B release; and killed a B cell clone bearing an anti-FVIII BCR. This killing was highly specific as B cells that did not express the anti-FVIII BCR were not killed. This may explain why Fc-FVIII has been reported to be more effective in tolerizing hemophilia A patients with inhibitors than unfused FVIII products. In future studies, we will explore the potential of Fc-proteins to target and kill unwanted B cells, especially in the context of protein replacement therapy and inhibitory antibody development.

Conclusions: Fc-FVIII can activate NK cells to kill anti-FVIII bearing B cells. This approach may be useful in preventing or treating patients undergoing protein replacement therapy with inhibitors.

Poster Board No. 58

New Treatment Modalities

New Approaches for Enzyme Replacement Therapy of Lysosomal Diseases with High Immunogenicity by Mass Spectrometric Identification of Antibody Epitopes

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Enzyme replacement therapies (ERT) have been successfully introduced for a number of Lysosomal Storage Diseases (LSDs) such as Gaucher (GD), Fabry (FD), and Pompe (PD) Disease. While effective for several LSDs, substantial problems can be caused by development of high immunogenicity. Patients who develop antibodies upon ERT can have allergic reactions, from mild symptoms to life threatening events. IgG antibodies may neutralize the lysosomal enzyme and prevent successful treatment. Here we report new therapeutic approaches by mass spectrometric identification and affinity characterization of antibody epitopes upon ERT, using synthetic epitope peptides that block antibodies directed against the infused enzyme. Therapeutic intervention using epitope peptide derivatives of low toxicity is expected to block neutralizing antibodies and substantially improve efficiency and safety of ERT. Identification of antibody epitopes from blood from FD and PD patients was obtained by a combination of proteolytic affinity- mass spectrometry and SPR biosensor analysis (SPR-MS). The epitope(s) from anti-alpha-galactosidase antibodies immobilized on a sepharose microcolumn were identified by trypsin digestion (2 hrs). The proteolytic peptide mixture was loaded onto an SPRMS interface; after washing out nonbinding peptides, the epitopes were eluted with 0.1 % TFA into the MS. The SPRMS combination was successfully applied to the epitope elucidation and affinity characterization of antibodies against alpha-galactosidase in 3 FD patients, and provided identical peptide sequences, αGal(309-332). The epitope (309-332) was synthesized by solid phase peptide synthesis (SPPS), and purified by reversed phase HPLC. SPR Analysis provided high affinity to the antibody (K_D , 39 nM). For cell culture studies skin fibroblasts are used to evaluate the epitope peptides on the uptake of lysosomal enzymes in the presence of the patient´s antibodies. The results showed that antibodies were blocked by tight binding to the epitope peptide, thus opening a new concept to reconstituting therapeutic efficiency of ERT.

Poster Board No. 59

New Treatment Modalities

Resorting the Function of the Colorectal Cancer (CRC) Gatekeeper Gene - Adenomatous Polyposis Coli (APC)

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Familial adenomatous polyposis (FAP) is a rare, inherited diseases caused by mutations in the adenomatous polyposis coli (APC) gene. FAP occurs in around 1/8,300 birth, it manifests equally in both sexes and is characterized by the development of hundreds to thousands of adenomas in the rectum and colon during the second decade of life.

A large number of the APC mutations in FAP patients are nonsense mutations that lead to introduction of pre-mature stop codons and APC loss of function.

Our goal was to test the feasibility and effectiveness of APC nonsense mutation read-through as a potential chemo-preventive therapy. Ten FAP patients harboring APC nonsense mutations were treated with the read-through inducing antibiotic erythromycin for 4 months. Endoscopic assessment of the adenomas was performed at baseline, after 4 and after 12 months. Adenoma burden was documented in terms of adenoma number, maximal polyp size and cumulative polyp size per procedure. Tissue samples were collected and subjected to molecular and genetic analyses.

Our results show that in the majority of patients the treatment led to a decrease in cumulative adenoma burden, median reduction in cumulative adenoma size and median reduction in adenoma number. Molecular and genetic analyses of the adenomas revealed that the treatment led to a reduced number of somatic APC mutations, reduced cellular proliferation and restoration of APC tumor-suppressing activity. Together, our findings show that induced read-through of APC nonsense mutations leads to promising clinical results and should be further investigated to establish its therapeutic potential in FAP and sporadic CRCs harboring nonsense APC mutations.

Currently we are launching an additional clinical trial using a different read-through agent and have gained new molecular insights into the mechanism of nonsense mutation read-through.

Poster Board No. 60

New Treatment Modalities

The High-Throughput Screening and The NMN as Models of Potential Therapies for Mitochondrial Diseases

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Mitochondrial diseases are a large family of genetic disorders characterized by defects in multiple biochemical pathways and cellular processes in mitochondria. It is associated with a decrease in energy production and other essential functions not related to ATP synthesis, making it challenging to identify therapeutic procedures. We have afforded two therapeutics strategies with *Saccharomyces cerevisiae* as model, one specific for coenzyme Q deficiency and a second one for general mitochondrial defects.
The first strategy consists of a High-Throughput Screening (HTS) to detect small soluble molecules bypassing the defect in the synthesis of CoQ_6 in yeast. This method requires the stabilization of the CoQ biosynthesis complex (Q-synthome) by *COQ8* gene overexpression and monitoring the growth in a non-fermentable carbon source such as glycerol. The application of this pilot approach allowed us to explore a library of 1200 natural microbial extracts, finding nine positive ones and identify five molecules with therapeutic potential in coenzyme Q_{10} human deficiency.

The second strategy analyzes the potential benefits of modifying NAD^+ levels by supplementation with its precursor, the nicotinamide mononucleotide (NMN). NAD^+ has described as a regulator of mitochondrial metabolism and has emerged as a new strategy of therapy for mitochondrial diseases. We have studied the effect of NMN on mitochondrial metabolism and the possible pathways involved. Our results showed that NMN supplementation induces mitochondrial responses that depend on mitochondrial sirtuins activation Hst4. This activation drives changes of proteins associated with mtDNA-nucleoids; increasing the mitochondrial activity by the expression of mtDNA-encoded proteins.

Poster Board No. 61

New Treatment Modalities

Phosphorylated Glucocerebrosidase: Next-Generation, Cost Effective ERT for Gaucher Disease

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Background: Existing ERTs for GD type 1 are insufficiently effective for alleviating cardiac, bone and lung symptoms. Neither are they adequate to treat neuronopathic forms of GD because the enzymes do not cross the BBB, and even if they would, are insufficiently targeting neuronal cells.

Objective: Oxyrane aims to produce a more stable β-glucocerebrosidase enzyme rich in M6P that targets a broader cell-type population, thereby alleviating remaining unmet needs in GD type 1 and unmet needs in GD type 2/3. Oxyrane employs 2 administration routes: intravenous approach to treat systemic symptoms of GD type 1, and intracerebroventricular approach to treat both neurological and systemic symptoms of GD type 2/3.

Method: Oxyrane uses yeast-based technology to augment the phosphorylation of N-glycans independently of the enzyme's 3D structure. Consequently, the OxyGCase variant is rich in M6P and shows a 50-fold increased M6P-mediated uptake in neuronal cells compared to imiglucerase.

Results: Studies in a D409V KI mouse model show that OxyGCase is efficiently targeting brain cells in various brain regions after intracerebroventricular administration, shown by enhanced activity levels and increased substrate reduction. Similar results are observed in peripheral organs, both via intravenous and intracerebroventricular administration. OxyGCase outperforms imiglucerase with respect to activity levels and substrate reduction, both via the intravenous and intracerebroventricular approach.

Conclusion: OxyGCase was proven to be efficacious (preclinical POC), safe and tolerable (6-month NHP toxicology study). Clinical POC of OxyGCase is planned for both administration routes. In addition, attractive Cost of Goods allow for a significant sale price reduction as next-generation GD type 1 treatment.

Poster Board No. 62

Raising Awareness for Rare Diseases

Glycogen Storage Disease type IIIA Patients Associated with Hematologic Malignancies

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Introduction: Glycogen storage disease type-IIIa (GSD-IIIa) is the most common subtype and manifests with liver, muscle involvement, and hypertrophic cardiomyopathy. In infancy/early childhood, liver involvement presents as ketotic hypoglycemia, hepatomegaly, hyperlipidemia, and elevated hepatic transaminases. In adolescence and adulthood, the liver disease becomes less prominent.

Case Report: The first patient was 26-years-old-male, who was referred to the hospital with hepatosplenomegaly at 16-months of age and diagnosed as GSD-IIIa with a homozygous mutation. On account of his swelling of knee and leukocytosis at 25 years of age, a bone marrow aspiration was performed and revealed the diagnosis of chronic myelocytic leukaemia. The second patient`s first symptom was hypoglycemic convulsion occurred at the age of 18 months. Additionally, she was diagnosed with GSD-IIIa at 35 years of age during the relapse of non-Hodgkin-lymphoma, which was diagnosed incidentally following parathyroidectomy owing to a parotid stone. The third patient was a four-years-old-girl. Although GSD was suspected at six months old due to hepatomegaly and hypoglycemia, she was diagnosed with GSD-IIIa and ALL-L2 together at the age of 4 when hepatosplenomegaly and lymphadenopathy were detected. The patient passed away in the second month of chemotherapy. Homozygous mutations detected in the AGL gene of the patients were [(p.Q667*)(c.1999CT)],[(p.Q1376*)(c.4126CT)],[(p.D251Efs*23)(c.753_756delCAGA)], respectively.

Conclusion: Despite G-CSF was shown as a significant cause of leukaemia in GSD-Ib before, as far as we know, our patients are the first GSD-IIIa cases reported with haematological malignancies in the literature. Believing this should be more than a coincidence, we do want to create awareness about hematologic malignancies and stimulate new studies to demonstrate the underlying etiological factors in GSD-IIIa.

Poster Board No. 63

Raising Awareness for Rare Diseases

Is Awareness of Inherited Metabolic Diseases Rising Progressively with Years of Education of Medical Students?

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Background: Inherited metabolic diseases (IMDs) are not sufficiently included in the national core-training program of medical faculties in Turkey, although a significant disease burden affects large proportion of the population due to high rate of consanguineous marriages. Vertical integration between basic and clinical sciences for biotinidase deficiency and phenylketonuria with real cases are discussed in 1st grade. In 3rd and 4th grades, only 5 theoretical classes exists for IMDs in medical curriculum of Çukurova University.

Objective: Our aim is to determine the awareness of IMDs among medical students throughout their education.

Method:13 closed questions were directed at medical students by their peers between 1st-6th grades.

Results: 267 medical students were included. 83(31%) were 1st, 54(20.2%) were 2nd, 40(14.6%) were 3rd, 41(15.4%) were 4th, 16(6%) were 5th, and 33(12.7%) were 6th grades. Overall, the rate of correct answers ranged between 16,3% and 95,9%. Statistically significant increase was observed for correct answers about probability of IMDs in adulthood as well as hyperglycemia as a symptom of IMDs with progressing grades (p0,05). Surprisingly, a negative correlation between progressive grades and awareness about content and timeframe of NBS was detected (p0,05).

Conclusion: Interestingly in contrast to expectations, we didn`t observe a significant rise of awareness in IMDs from first to sixth grades, contrarily, there is a partial decrease with progressive classes. So, current medical education may not provide adequate training of IMDs. To ameliorate this situation, the students suggest us to perform not only theoretical classes but also case-based integrated sessions with small groups and more practice in metabolism departments with real cases of IMDs throughout their education.

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Poster Board No. 64

Rare Syndromes

Clinical Whole-Genome Sequencing, Co-Occurring Rare Diseases and Pharmacogenetic Profiling

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Background: In the current genomics era, interpretation of high-throughput sequencing data constitutes the main bottleneck on the path to accurate diagnosis of Mendelian disorders. Large-scale reference cohorts such as ExAC/gnomAD are valuable for the population-frequency-based filtering of the myriad of detected sequence variants.

Objective: Using the example of fibrillinopathies, such as *FBN1*-related Marfan syndrome (MFS) and *FBN2*-related congenital contractural arachnodactyly (CCA), we assessed the frequency and co-occurrence of pathogenic *FBN1*/2 sequence variants in ExAC/gnomAD and the largest Swiss database of Marfan genomes, respectively.

Methods: By focusing on *a priori* pathogenic sequence variants in ExAC/gnomAD, we calculated conservative prevalence for MFS and CCA. In addition, we screened whole genomes (60× WGS, PE150) of ~550 patients with rare (aortic) disorders for pathogenic and functional sequence variants in *FBN1, FBN2*, and 12 pharmacogenes, respectively.

Results: We show the presence of pathogenic *FBN1/2* variants in the apparently healthy reference cohort ExAC/gnomAD, providing prevalence estimates for MFS and CCA. In our Swiss cohort, we identified two families with dual *FBN1* and *FBN2* mutations, explaining the variable phenotype within these families including clinical features of MFS and CCA. In one of these families, we also detected a pharmacogenetically actionable variant in a drug metabolizing enzyme.

Conclusions: Our results not only demonstrate that apparently healthy reference data sets may include individuals with late-onset or unrecognized disease, but also show that fibrillinopathies occur more frequently than expected and may co-occur. Furthermore, we emphasize the importance and increasing possibility of detecting digenic and pharmacogenetically relevant sequence variants using WGS.

Poster Board No. 65

Rare Syndromes

From Diagnosis to Therapy: Novel Approach Reveals Celiprolol as Medical Therapy of Choice for Vascular Ehlers-Danlos Syndrome

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Background: Patients with the rare connective tissue disorder vascular Ehlers-Danlos syndrome (vEDS) are at increased risk for fatal aortic ruptures. Using a mouse vEDS model, we established an objective read-out system for the assessment of the clinically highly relevant biomechanical integrity of the thoracic aorta.

Objective: By means of this novel read-out system, we aimed to assess the effects of antihypertensive drugs on the biomechanical integrity of the weakened murine vEDS thoracic aorta as potential medical therapy in vEDS.

Method: Mice modelling vEDS were treated with the beta-blockers celiprolol (Selectol®) or bisoprolol (Bilol®) or the ARB losartan for 4 weeks. 1.5-mm-long sections of the ascending and descending murine thoracic aorta were mounted on a tissue puller and uniaxially stretched until rupture while recording the tensile force (in mN).

Results: The rupture force was significantly lower in untreated heterozygous compared to wild-type mice and decreased with increasing distance from the heart for both heterozygotes and wild-types. We showed that celiprolol but neither bisoprolol nor losartan increased the rupture force of the thoracic aorta in heterozygous mice (PMID: 31056650 and 31693161).

Conclusions: Our novel read-out system is suitable for detecting significant differences in the rupture force of the murine thoracic aorta and allows the assessment of the effect of candidate drugs on the biomechanical integrity of the aorta. Although the added value of other antihypertensive drugs in vEDS, if any, is unknown, celiprolol, but not losartan and bisoprolol, is currently the medical therapy of choice for vEDS, until further evidence emerges.

Poster Board No. 66

Rare Syndromes

Klippel-Trenaunay-Weber Syndrome Without Cutaneous Haemangioma: A Case Report

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Background: Klippel-Trenaunay-Weber (KTW) syndrome is a rare congenital disorder which is usually presented as a triad of symptoms: cutaneous haemangioma ("port-wine stain"), lymphatic anomalies and venous varicosities associated with soft tissue hypertrophy, most frequently on the lower limb, and less commonly on the head, upper limb and trunk.

Objective: The main aim of this case report is to present a clinical presentation and treatment of a patient with KTW syndrome with vein malformation on a right forearm as rare localisation and in absence of cutaneous haemangioma.

Case report: A female newborn on the first clinical examination after labour was presented by a painless swelling of the right forearm and vein varicosities which persisted during developmental age, as well as progressive motion range reduction, with no noticeable cutaneous haemangioma. At the age of 9, child was presented with the pain in the right hand, and the echotomography showed venous malformation with suspected arteriouvenous (AV) fistula. In order to determine the presence of fistula the CT scan, flebography and pulse oscilloscopy was recommended, which verified the slow-flow venous malformation without AV fistula. In the next six years symptoms were causing severe disabilities, so the surgical treatment was repeated annually in next three years and also a year ago, when it was described and recognized as a part of Klippel-Trenaunay-Weber syndrome. In order to maintain patient`s quality of life, further surgical treatment of advanced vascular anomalies is planned.

Conclusion: Klippel-Trenaunay-Weber syndrome is a rare syndrome which may be progressive and cause severe disabilities and quality of life impairment, especially when it is not early recognized, due to unusual clinical presentation. In our country, currently there is only surgical treatment recommended in these cases, but in advanced cases other therapy options should be considered.

Poster Board No. 67

Rare Syndromes

Mass Spectrometry Imaging (MSI) Identifies Changes in The Region-Specific Distribution of Cerebral Lipids in A Mouse Model of Niemann-Pick-disease Type C1 (NPC1)

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NPC1 is a rare autosomal-recessive lipid transport and storage disorder in which the brain becomes affected by a selective, progressive dysmyelination and distinct neurodegeneration, but detailed information on affected functional regions is still lacking. We used frozen brains of *NPC1−/−* and control wild type male and female mice (n=10; 60-70 days old) as approved by governmental authorities (study-no. 7221.3-1-01-011/16). Frontal brain sections (10 µm) at 5 different levels were analyzed by Matrix assisted laser desorption/ionization mass spectrometry imaging (MALDI LTQ-Orbitrap XL mass spectrometer at 100 µm lateral resolution) followed by cytoarchitectonic evaluation. SCiLS Lab MVS, version 2018, was used to identify discriminative peaks and generate MS images. Lipids were assigned from LIPID MAPS (http://www.lipidmaps.org) based on the high accuracy mass measurement. From the 103 lipids which were identified in positive and negative ion mode analysis, 57 appeared altered in *NPC1−/−* brains. GM2 (d18:1/18:0) and GM3 (d36:1) levels were significantly increased in multiple regions in *NPC1−/−* such as cerebral cortex but with highest levels in distinct subcortical regions e.g. amygdala, hippocampus (CA3) and olfactory bulb. Also significant region-specific accumulation of ganglioside GM1(d38:1) and phosphoinositol (PI 36:4) occurred with highest levels of GM1 confined to retrosplenial cortex, thalamus, cerebellar grey matter and for PI (36:4) in addition, in olfactory bulb, piriform cortex and corpus callosum but not in cerebellum. In contrast to the gangliosides, sulfatides (ST), such as ST (d18:1/24:1) levels were significantly reduced in several distinct forebrain regions except in cerebellar white matter in *NPC1−/−* brains. This indicates a wider range of cerebral lipid changes in brains of *NPC1−/−* mice which allows a functionally analysis and interpretation with specified behavioral tests and provides foundation for studying the target and off-target effects of therapeutic interventions in animal models.

Poster Board No. 68

Rare Syndromes

Vesicle Tethering Complexes Control the Fate of Golgi Glycosylation Enzymes

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Background: The Golgi is an essential hub in the secretory pathway where cargo and glycosylation enzymes are delivered to undergo modifications before targeting to the final destination. The Conserved Oligomeric Golgi (COG) complex, a multisubunit tethering complex, ensures Golgi homeostasis by orchestrating retrograde vesicle trafficking within the Golgi. The four subunits Golgi Associated Retrograde Protein (GARP) complex controls the retrograde transport from endosomes to the trans-Golgi network. Human COG defects lead to a set of multi-systemic diseases - COG-Congenital Disorders of Glycosylation (COG-CDG) and Saul-Wilson syndrome. Diseases associated with GARP complex include Pontocerebellar hypoplasia and Amyotrophic Lateral Sclerosis 1.

Objective: To create a human cellular model to investigate the effect of COG and GARP knock-outs (KO) on the Golgi glycosylation machinery.

Method: The CRISPR approach was utilized to generate HEK293T and RPE1 cells deficient for COG and GARP subunits as well as for double KO (DKO) combinations. Flow cytometry, EM, RUSH pulse-chase approach, and superresolution microscopy were used.

Results: COG and GARP KO cells show defects in retrograde trafficking, sorting, and glycosylation. COG KO cells also show fragmentation of the Golgi and an enlargement of the endolysosomal compartment. Glycosylation and trafficking defects were more severe in COG/GARP DKO cells. The stability of MGAT1, B4GALT1 and ST6GAL1 enzymes was compromised in both COG and GARP KO cells. The RUSH approach revealed that the localization of Golgi enzymes was altered in KO cells.

Conclusion: Our results demonstrated that both COG and GARP vesicle tethering complexes are essential for the maintenance of Golgi glycosylation machinery in human cells.

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Poster Board No. 69

Rare Variants of Rare Diseases

Clinical Evolution of An Intermediate Type 2-3 Gaucher Patient

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Aim: The presentation of the clinical evolution for a Gaucher Disease patient, with genetic double mutation [D409H; H255Q], classified as intermediate type 2-3 GD

Background: Gaucher disease (GD) is an autosomal recessive lysosomal storage disease, which is mainly due to mutations in the GBA gene. Most of the mutant alleles described so far bear a single mutation. However, there are a few alleles bearing two or more DNA changes. It has been reported that patients homozygous for the [D409H;H255Q] (p.Asp448His:p.His294Gln) double mutant allele, present a severe type 2 neurologic Gaucher disease.

Case report: Our case is a 5 year-old boy. His parents have Albanian origin without consanguinity. Pregnancy and delivery were normal. Birth weight was 2.9 kg. At the age of 4 months, he was referred to our service because of hepatosplenomegaly and thrombocytopenia**.** At the age of 7 months, enzyme activity of glucocerebrosidase resulted very low and the level of chitotriosidase was high, confirming the diagnosis of Gaucher disease. Genetic testing identified the presence of homozygote double mutation [D409H;H255Q]. Enzyme replacement therapy with imiglucerase, every other week has been started. At the age of 15 months, this boy presented neurological signs, including neck rigidity and ocular movements disorders. The neurological signs became more evident and during the next visit, at the age of 20 months, the child presented: generalized dystonia, swallowing difficulties, chest deformity (Fig.1), oculomotor apraxia, extrapyramidal syndrome, coordination impairment. During the follow up period, the child showed good improvements of visceral and hematological signs, together with the stability of chitotriosidase and lysoGB1 biomarkers, until the age of 3 years old. During the fourth year of life, the level of lysoGB1 were rising (Fig.3). At this time,we started ambroxol-chaperone therapy. During last two years, the neurological status of the child was complicated by hydrocephalus (Fig.2) and seizures. Due to swallowing difficulties, we carried out a percutaneous gastrostomy, which preceded a ventriculo-peritoneal shunt, performed one week later. Neurological impairment remains catastrophic, despite the treatment with ERT, ambroxol, levetiracetam and baclofen.

Discussion: A double mutation [D409;H255Q] is present in approximately 50 % of Albanian GD patients. Most of our patients have a heterozygous form, combined with N370S (type1 GD) and rarely combined with L444P or F213I, leading to neuropathic forms,with a variable gravity. Homozygous form leads to severe neurological impairments, such as type 2 GD [Michelakakis et al., 2006], dying within the first or second year of life, meanwhile another study reported a case, such as an intermediate phenotype 2-3 [Filocamo et al., 2005], surprisingly of Albanian origin. Another publication [Swati Sathe et al 2008] describes another Albanian patient, with double mutation [D409;H455Q], who died at the age of 31 months, presenting neurological impairment and hydrocephalus. Our case presented neurological signs, before the age of two

years old. Initially we thought for Gaucher type 2 but he continues to survive, for three more years. Genotype-phenotype correlations completed the criteria, for an intermediate form 2-3 Gaucher disease type. According to the literature [Ellen Sindrasky et al.,2010] some patients with intermediate form 2-3, present severe neurovisceral manifestations, during infancy or early childhood but they survive past the second year of life, with death occurring in mid childhood (age 3-7 years old). Under the treatment with ERT, our patient has had a slow evolution till the age of 3 years old.. Now the patient is 5 years old and alive, despite severe neurological impairment. This case is another example that GD is a continuum of phenotype.

Conclusion: Intermediate type 2-3 Gaucher disease presents a slower clinical evolution compared to the type 2, nevertheless the neurological impairment remains life threatening, despite the treatment.

Poster Board No. 70

Rare Variants of Rare Diseases

First Case Report of Gaucher Disease and Graves' Thyroiditis

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Introduction: Gaucher disease (GD) is the most common storage disorder caused by the deficiency of glucocerebrosidase. It is an autosomal recessive disorder that primarily affects the mononuclear phagocyte system by lipid accumulation. The worldwide prevalence is 1/60 000. Although patients typically manifest with hepatosplenomegaly, hematological changes like, anemia and thrombocytopenia together with orthopedic complications, it is well known that autoimmune diseases are more frequent. Graves' thyroiditis is a systemic autoimmune disorder characterized by the infiltration of thyroid antigen-specific T cells into thyroid-stimulating hormone receptor (TSH-R) expressing tissues. Pathogenesis of Graves' thyroiditis is complex and includes both genetic and environmental factors. It is much more frequent in women and associated with cardiovascular, ophthalmologic and other systemic manifestations.

Case Report: Here we report a 48 years old female patient diagnosed as Graves' disease who followed-up with GD for 10 years and had been receiving enzyme replacement therapy. She admitted to our clinic with palpitation and insomnia out of routine controls. Laboratory evaluation put forward Graves' thyroiditis with thyroid-stimulating hormone (TSH):

Discussion: Previous studies have shown that autoantibodies are more frequent in type 1 Gaucher disease when compared with healthy population. However, they are not associated with an increased prevalence of clinically active autoimmune diseases, except some rare cases of autoimmune hemolytic anemia, antiphospholipid syndromes, and immune thrombocytopenia. No significant association of GD1 with other autoimmune diseases, such as Graves` disease, Hashimoto disease or systemic sclerosis have been reported in previous studies. To the best of our knowledge, our case is the first report of Graves' thryroiditis in GD. Whether this association is accidental or dependent on autoimmunity were commend that all GD patients be investigated.:

Poster Board No. 71

Relations Between Rare Diseases and Common Disorders

Role of Cdk5rap2 in Neocortical Inhibition and Excitation Balance

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Autosomal recessive primary microcephaly type 3 (MCPH3) is characterized by congenital microcephaly and intellectual disability. Further features include hyperactivity and seizures. The disease is caused by biallelic mutations in the Cyclin-dependent kinase 5 regulatory subunit-associated protein 2 gene *CDK5RAP2*. In the mouse, *Cdk5rap2* mutations similarly result in reduced brain size and a strikingly thin neocortex already at early stages of neurogenesis that persists through adulthood. The microcephaly phenotype in MCPH arises from a neural stem cell proliferation defect. Here, we report a novel role for Cdk5rap2 in the regulation of dendritic development and synaptogenesis of neocortical layer 2/3 pyramidal neurons using a combined morphological and electrophysiological approach.

Poster Board No. 72

Role of Registries and Big Data

RaDiCo (Rare Disease Cohorts): A Platform for Rare Disease (RD) E-Cohorts

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Background: Implementing RD cohorts is a challenge since RDs are seldom, often underdiagnosed and spread over the national territories.

Objective: To create a national platform dedicated to the development, within a research framework, of RD e-cohorts meeting strict criteria of excellence.

Material and Methods: The RaDiCo program coordinated by Inserm comprises an operational platform and several RD e-cohorts. The platform, built on the "cloud computing" principle, is oriented as an “Infrastructure as a Service”; Interoperable; In compliance with the General Data Protection Regulation; Within a Certified Health Data Host; Ensuring continuous monitoring of data quality and consistency; In line with the French Health Data Hub.

Results: 13 e-cohorts projects covering 67 RDs have been selected through a national call and launched (2017). Depending on cohorts, they aim at: Describing RDs’ natural history; Establishing phenotype-genotype correlations; Deciphering RDs’ pathophysiology; Identifying new therapeutic avenues; Assessing RDs’ societal and medico-economic impact; Identifying patients eligible for new therapeutic approaches. As of June 15, 2019, 4035 patients have been included and 1560 were eligible to come (recruitment target 97%); 26 publications appeared in international peer-reviewed journals.

Discussion: Several secondary objectives of the e-cohorts have been reached. Member of the 3rd RDs’ National Plan, RaDiCo is tightly linked to patients’ associations and advocacy groups. Partnerships with industry confirm RaDiCo’s sustainability. The cohorts’ PIs are involved in 10 European Reference Networks. RaDiCo participates to the RD-European Joint Program.

Conclusion: RaDiCo provides a flexible and easily sharable platform enabling the creation and follow-up of national/international RD e-cohorts.

Poster Board No. 73

Role of Registries and Big Data

The Rare Disease Cohorts (RaDiCo) Program: Set Up and Follow-Up of National and International E-Cohorts

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Background: Rare Disease (RD) professionals have highlighted the critical need to implement national/international, multidisciplinary, high-quality cohort studies to address key scientific and medico-economic questions.

Objective: To implement RD e-cohorts within a research framework, supported by an interoperable platform of mutualised expertise, resources and services, with shared standardised processes and tools.

Material and Methods: The RaDiCo program coordinated by Inserm has launched 13 national/ international e-cohorts, covering 67 RDs, selected through a national call. Depending on cohorts, they aim at: Describing RDs' natural history; Establishing phenotype-genotype correlations; Deciphering RDs' pathophysiology; Identifying new therapeutic avenues; Assessing RDs' societal and medico-economic impact; Identifying patients eligible for new therapeutic approaches.

Results: The cohorts cover the following RDs: Congenital defects of the eye, Still`s disease, Low Phospholipid-Associated Cholelithiasis syndrome, Cystinosis, Alport syndrome, Skin RD burden, Genetics of Intellectual Deficiency and Autism Spectrum Disorders, Imprinting disorders, Mucopolysaccharidoses, Primary Ciliary Dyskinesia, Periodic Paralysis, Idiopathic Interstitial Pneumonia, and Vascular Ehlers-Danlos Syndrome. As of June 2019, 4035 patients have been included and 1560 were eligible to come (recruitment target 97%);

Discussion: RaDiCo assets are the following: A flexible, interoperable and easily sharable platform enabling the inclusion of new cohorts within an industrialization framework. Several secondary objectives of the cohorts have already been reached and published. RaDiCo cohorts are involved in 10 European Reference Networks and in the RD-European Joint Program. Sustainability is based on academic resources and several partnerships with industry.

Conclusion: RaDiCo developed e-cohorts and industrialized the process that enables including new RD cohorts either national or international.

Poster Board No. 74

Single Gene Diseases

Differential Gene Expression Patterns and Functional Analysis of Skin Samples from a Patient with Progressive Osseous Heteroplasia

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Background: Progressive osseous heteroplasia (POH) is an ultra-rare genetic disorder, characterized by progressive extra-skeletal bone formation. It is caused by heterozygous mutations in the *GNAS* gene, that is involved in multiple signaling transduction pathways and functions. We studied two monochorionic twin sisters of 7 years old diagnosed with POH, sharing the same *de novo* pathogenic mutation in *GNAS* but with very different clinical manifestations of the disease. While one of the sisters shows an aggressive and disabling disease, the other presents an asymptomatic phenotype.

Objective: We hypothesize that mechanisms controlling the gene expression might be under the different phenotypes showed by both affected twins. We analyzed gene expression patterns in skin samples from the affected twin to elucidate genes and pathways implicated in ectopic bone formation that could be used as potential therapeutic targets.

Method: We carried out a differential expression analysis followed by a functional analysis using 770 genes from 13 canonical signaling pathways in three affected skin samples from different locations compared with three skin samples from healthy subjects using nCounter technology (Nanostring).

Results: We found 24 differentially expressed genes comparing affected with normal skin, most of them under-regulated. We also detected different levels of gene expression dis-regulation between affected samples. Functional analysis revealed some pathways related to bone metabolism, like Wnt among others, under-regulation of G alpha (q) signaling events and serotonergic synapse, and up-regulation of genes implicated in epithelial-mesenchymal transition events.

Conclusion: We identified several genes and pathways implicated in the development of ectopic bone formation in POH.

Poster Board No. 75

Single Gene Diseases

GNAS Methylation Pattern and Epigenome Analysis in Two Monozygotic Twins with Progressive Osseus Heteroplasia but Different Clinical Phenotype

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Background: Progressive Osseus Heteroplasia (POH) is a rare genetic disorder characterized by progressive heterotopic ossification. It is caused by inactivating mutations of one of the most complex locus of the human genome, the GNAS gene, which shows genetic imprinting and encodes the α-subunit of the stimulatory G protein that plays a key role in several signalling pathways, including those related to bone formation.

Objective: Assuming that DNA methylation affecting gene expression can modify the phenotypic development of POH, the main objective of this study is to investigate the underlying clinical and molecular factors implicated in the unique case of a couple of monozygotic twins with the same GNAS inactivating mutation, but displaying a completely phenotypic discordance: the first twin develops an aggressive form of the disease while her sibling is almost asymptomatic.

Method: The global blood DNA Methylation patterns of both twins affected by POH was investigated through an epigenome whole association study (EWAS) using the microarray technology from Illumina (MethylationEPIC BeadChip; 850K). The evaluation of the methylation status of the complex locus GNAS1 was performed using the Methylation-Specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA).

Results: DNA methylation analysis showed some interesting differences in the methylation pattern in both twins that affect gene expression in several signalling pathways that could explain the phenotypic discordance of the present case.

Conclusion: Further studies on gene expression patterns in different tissues are necessary to completely investigate the differential onset of the disease.

Poster Board No. 76

Single Gene Diseases

Lipin1 deficiency: A Novel Mutation in A Patient with Recurrent Rhabdomyolysis

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Background: Lipin-1 deficiency is an autosomal recessive disorder due to mutations in *LPIN1* gene. The clinical presentation is severe with recurrent rhabdomyolysis, occurring especially in early childhood often triggered by febrile illness, and less commonly by exercise and fasting. Creatine kinase (CK) levels can rise above 10,000 IU/L. Renal failure may occur and autopsies of patients revealed cardiomyopathy and hepatosteatosis. Development is normal between episodes. Diagnosis is made by molecular analysis. Treatment is symptomatic.

Case Presentation: A 6-year-old male presented with myalgia due to recurrent rhabdomyolysis. His parents were consanguineous. His cousin had Duchenne Muscular Dystrophy. The first episode occurred at the age of 18 months following an acute gastroenteritis. Second episode occurred after a prolonged hypoglycemia due to a gastroenteritis at 4 years of age and, CK levels were above 200 000U/L. Between the episodes, CK concentrations were mildly elevated. Growth parameters and neurodevelopmental milestones were appropriate for his age. Physical, neurological and cardiological evaluation did not reveal any abnormality. Urine organic acid and acylcarnitine profile were normal. Electromyography showed myogenic pattern. Intramyocellular lipid deposition was observed on muscle biopsy. Molecular analyses for myophosphorylase deficiency and carnitine palmitoyltransferase-2 deficiency detected any mutation. Genetic analysis for *LPIN1* gene put forward a novel, homozygous missense p.D481H (c.1441G C) mutation.

Conclusion: Up to 60% of patients with unexplained recurrent rhabdomyolysis in early childhood who have normal acylcarnitine profile are shown to have lipin-1 deficiency. We report this case, as a novel mutation was detected and there were two different inherited cause of rhabdomyolysis in the same family.

Poster Board No. 77

Single Gene Diseases

Therapeutic Targeting of mTOR Pathway in a Severe Case of Progressive Osseous Heteroplasia

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Background: Progressive osseous heteroplasia (POH) is an ultra-rare genetic disorder characterized by an inactivating mutation in the GNAS gene that causes heterotopic ossification. Inhibition of mammalian target of rapamycin (mTOR) signaling pathway has been proposed as a therapy for progressive bone fibrodysplasia and non-genetic forms of bone heteroplasia. Herein we describe our experience with the use of Everolimus in the rescue therapy of an identical twin girl suffering an aggressive clinical phenotype of POH.

Methods: Clinical evaluation of the progression of the disease during Everolimus treatment was performed periodically. Cytokine markers involved in bone metabolism, as well as protein markers, related to the bone, activity was analyzed to explore bone turnover activity.

Results: The patient received Everolimus therapy for 36 weeks. During treatment, no clinical improvement of the disease was perceived. We could observe that biochemical parameters as bone turnover activity was significantly reduced, namely β-CTX ($r^2 = -0.576$, P-value = 0.016) and PNIP ($r^2 = -0.598$, P-value = 0.011). Additionally, bone metabolism biomarkers evidenced only a significant positive correlation with PTH levels.

Conclusions: Everolimus treatment did not modify the clinical progression of the disease in an aggressive form of POH, although an impact in the protein markers studied was observed.

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Single Gene Diseases

Tissue-specific Autoimmunity Controlled by Aire, a Gene Responsible for APECED

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Tissue-specific autoimmune diseases are assumed to arise through malfunction of two checkpoints for immune tolerance: defective elimination of autoreactive T-cells in the thymus, and activation of these T-cells by corresponding autoantigens in the periphery. However, evidence for this model and the outcome of such alterations in each or both of the tolerance mechanisms have not been sufficiently investigated. We studied these issues by expressing human AIRE (huAIRE) as a modifier of tolerance function in NOD mice wherein the defects of thymic and peripheral tolerance together cause type I diabetes (T1D). Additive huAIRE expression in the thymic stroma had no major impact on the production of diabetogenic T-cells in the thymus. In contrast, huAIRE expression in peripheral antigen-presenting cells (APCs) rendered the mice resistance to T1D, while maintaining other tissue-specific autoimmune response and Ab production against an exogenous protein Ag, due to the loss of Xcr1+ DCs, an essential component for activating diabetogenic T-cells in the periphery. These results contrast with our recent demonstration that huAIRE expression in both the thymic stroma and peripheral APCs resulted in the paradoxical development of muscle-specific autoimmunity. Our results reveal that tissue-specific autoimmunity is differentially controlled by a combination of thymic function and peripheral tolerance, which can be manipulated by expression of huAIRE/Aire in each or both of the tolerance mechanisms.

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Single Gene Diseases

Two Monochorionic Twins with a Critically Different Course of Progressive Osseous Heteroplasia

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Background: Progressive osseous heteroplasia (POH; OMIM 166350) is a rare autosomal-dominant genetic disorder characterized by progressive heterotopic ossification. POH is one of the clinical manifestations of an inactivating mutation in the *GNAS* gene. *GNAS* alleles show genetic imprinting, produce several transcript products, and the same mutation may lead to strikingly different phenotypes. The complexity of POH makes that this disorder still lacks therapeutic options.

Objectives: To describe a unique case of POH in two monochorionic twins, who presented an almost asymptomatic versus a severe clinical course.

Methods: The progression of the disease in both cases and the treatment effectivity were evaluated with measuring of serum markers involved in osseous metabolism. We report the results of the therapeutic interventions currently available for heterotopic ossification in the patient with a severe course.

Results: Due to the rapid progression of the disease in the patient with severe course a treatment protocol was administrated: mecasermin, naproxen, tretinoin, oral retinoid, pamidronate, itraconazole, methylprednisolone, and indomethacin. All the chosen interventions failed in stopping the formation of ectopic bone.

Conclusions: The difference in the clinical course between two twins with POH could be because of a very complex molecular mechanism that goes beyond a GNAS mutation. We failed at finding any medication that could ameliorate the symptoms of POH, let alone halting the progression of the disease.

Table 1. Treatments received by the patient with severe clinical course of POH.

DRUG	DOSE	MECHANISM	ADMINISTRATION LENGTH	ADVERSE EVENTS	CAUSE OF DISCONTINUATION	REFERENCES
Mecasermin	0.04 mg/day	rhIGF-I	15 days	No	Worse serum markers; same clinical	(35) Ueland T,2005 (36) Cao X, 2011 (37) Guntur AR, 2013
Naproxen	100mg	NSAIDs	40 days	Aphtous ulcers	Aphtous ulcers	*used as painkiller and antinflammatory in chronic conditions.
Topical Tretinoin	0.10%	Retinoid: Stimulation of Gsα expression at a transcriptional level	10 days	Red, swollen rash in the chosen regions	Ossification over the scapula grew	(43) Chan SD,1990 (44) Shimono K, 2010 (45) Shimono K, 2011
	0.025%					
Oral acitretin	10 mg/day		1 month	No	Coalescence of bony spikes of the back and progression of the plate over the left scapula, as well as appearance of new spikes surrounding the abdominal plates.	(46) MA Zasloff, 1998
Pamidronate	2.5 mg/kg	Bisphosphonate: Slows the release of calcium, blocking the mineralization of the bone matrix	3 days	Worsened myalgia and asthenia and onset of low-grade fever	Manifestations of POH progressed	(48) P Schuetz, 2005
Itraconazole	6.6 mg/kg/q.d.	Antifungal: acts as a potent suppressor of the Hh signaling pathway	3 months		Biochemical markers of bone formation returned to previous levels, and absence of clinical improvement in the disease progression	(54) J Kim, 2010
	9.5 mg/kg/q.d.		1 month			
Methylprednisolone	20 mg/kg/q.d.	Corticosteroid hormone	5 days		Absence of clinical improvement in the disease progression, despite reuction of markers of bone formation after the initial bolus.	(21) Pignolo RJ, 2011 (24)) Morales A, 2002
	Slow tapering		6 months			
Indomethacin	3 mg/kg/b.i.d	NSAIDs	6 months		Currently on indomethacin.	(64) Vanden Bossche, 2005 (68) Athanasou NA, 1994
	4 mg/kg/b.i.d					