levels are positive on the 1st tier. 2nd tier testing of known immunodeficiency genes using the IonAmpliSeq PID panel on Ion Torrent PGM is performed to increase positive predictive value.
Results: Of the 720 children tested so far, the average level is 250 TREC/µl. 11 samples, all from NICU patients, have fallen below cutoff, without suspicion of a SCID diagnosis. In the retrospective project, screening samples from 7 patients with known immunodeficiency due to defects in IL2RG, JAK3, IL7R, PGM3, LIG4, RECQL4 and ADA had TRECs of 0, 0, 0, 11, 0, 60, and 0/µl, respectively. 2nd tier HTS was able to confirm the molecular alterations in 5,5 of the 7. The PID gene panel lacked coverage of the remaining mutations, and needs refinement before implementation.
Conclusion: TRECs as 1st tier, and HTS SCID gene panel as 2nd tier, promise rapid detection of newborns with SCID and other severe T-cell deficiencies.

P07.28
High-throughput multiplexed digital droplet PCR for detection of severe combined immunodeficiency in newborn screening

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Introduction: Measurement of T-cell receptor excision circles (TREC) is widely used for detection of Severe Combined Immunodeficiency (SCID) in newborn screening programs (NBS). Currently, qPCR is predominantly used to quantify TREC in dried blood spots (DBS). qPCR requires normalization to controls and is susceptible to variations in amplification efficiency. We evaluated digital droplet PCR (ddPCR) as a novel method for SCID detection.
Materials and Methods: DNA was extracted from one 3mm DBS punch using wash steps and denaturation at 99°C. Simultaneous quantification of TREC and RNAseP (internal control) was performed using AutoDG and QX200 ddPCR Bio-Rad system. A total of 10 diagnosed patient DBS (1 X-linked SCID, 1 RAG1 Omenn syndrome, 2 idiopathic T-cell lymphopenia (iTCL), 1 chr22q11.2 deletion negative TCL, 2 DiGeorge syndrome, 1 CHARGE syndrome, 1 ataxia telangiectasia and 1 cartilage hair hypoplasia) and 80 normal DBS (ages≤7days, gestational age≥36weeks) were screened for TREC levels. Results are expressed in TREC/ul blood.
Results: All previously diagnosed patient DBS were confirmed to contain ≤15 TRECs. Normal DBS ranged 46-276, average of 132 TRECs. Dilution experiments showed LLOQ at 14 TRECs. LOD was 11 TRECs. Precision experiments showed <20 %CV for intra-assay (at 54 TRECs) and inter-assay (at 60 TRECs) reproducibility. A reference range study is currently ongoing.
Conclusions: We have developed a highly sensitive and accurate multiplexed ddPCR method for absolute quantification of TREC in DBS without the need for standard curve. The method is cost-effective, high-throughput and suitable for NBS testing.

P07.29
Altered expression of DNA repair enzymes involved in molecular signaling pathway dependent on p53 in systemic lupus erythematosus

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Introduction: In systemic lupus erythematosus (SLE) apoptosis is thought to be defective, producing increased extracellular DNA and dsDNA antibodies. The p53 pathway is crucial in apoptosis regulation. We investigated if expression of enzymes (OGG1 and SIRT1) and transcription factor (FOXO1) involved in p53 pathway were altered in SLE, and associated with immune activation.
Material and Methods: We studied 32 SLE patients (10 with active lupus nephritis (LN), 12 with inactive LN, and 10 SLE in absence of LN); and 20 healthy controls. The mRNA levels for p53, SIRT1, OGG1 and FOXO1 were quantified by RT-qPCR in the urinary cellular pellet.
Results: Urinary mRNA levels of p53, OGG1 and FOXO1 were significantly diminished in active LN ($p<0.01$, $p<0.01$ and $p<0.05$, respectively), whereas in the other patient groups were similar to control group. However, the expression of SIRT1 in the urinary sediment had a significant increase ($p<0.01$) in active group compared to controls. Furthermore, the three patient groups showed an inverse correlation between FOXO1 and OGG1 mRNA levels with antibodies anti-dsDNA ($r=-0.47$ and $r=-0.55$, $p<0.01$), and Systemic Lupus Activity Index (SLEDAI) ($r=-0.22$ and $r=-0.41$, $p<0.05$). Whereas urinary expression of SIRT1 had a direct correlation with anti-dsDNA ($r=0.87$, $p<0.001$) in active LN.
Conclusions: These data show a transcriptional dysregulation in DNA repair enzymes SIRT1 and OGG1, and transcription factor FOXO1 involved in the p53 molecular pathway in SLE patients. In addition, mRNA quantification in the urinary sediment could be a non-invasive method to establish its association with the renal impairment in SLE.

P07.30
Screening of KLF1 gene variants in cases with increased levels of fetal hemoglobin

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Introduction: KLF1 is a transcription factor, promoting shift in expression from γ to β-globin. This process is carried out either directly, by activating transcription of β-gene, or indirectly by activating transcription repressors for silencing γ-genes. Variations of KLF1 lead to increased expression of fetal hemoglobin (Hb F), showing clinical benefits for thalassaemia patients, making it an ideal target for gene intervention to increase HbF levels and thus ameliorating the patient's phenotype.
Material and Methods: This study aimed to identify possible variations of KLF1 gene, in β-thalassaemia heterozygotes and normal controls presenting high levels of HbF>4%. DNA from 47 blood samples was obtained (32 heterozygotes/ 15 normal) and analyzed by PCR and Sanger sequencing of KLF1 gene.
Results: An already characterized alteration (-148G>A) detected for the first time in the Greek population (β-thalassemia heterozygote, HbF 19,5%) in coexistence with one published variation of unknown significance (c.544T>C,p.Phe182Leu). Two alternations, not yet recorded (g.1980C>A, c.831A>C), were found in β-thalassemia heterozygotes, (HbF: 5,9% and 10,1%) and a frequent polymorphism (c.304T>C,p.Ser102Pro) was present in the majority of tested samples. γ-genes investigation prior to this of KLF1 revealed the polymorphism -158C>T (HBG1, Cretan-type) for the first time in an Albanian origin sample.
Conclusions: KLF1 analysis in a small cohort of samples revealed variations that could possibly contribute in differential HbF expression. More extensive analysis of the above gene along with other non globin genes-modifying factors of HbF expression- has to be conducted especially in β-thalassaemia patients in order to unravel the mechanism of Hb F regulation.

P08 Intellectual Disability

P08.01
The clinical significance of 15q11.2 BP1-BP2 duplications: - Where do we stand?

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The 15q11.2-q13 region has been well characterized, being associated with a range of syndromatic copy number variants (CNVs), and comprises five established break points sites (BP1 to BP5).
While the clinical effect for BP1-BP3, BP2-BP3 and BP4-BP5 CNVs is well established, the same cannot be said for BP1-BP2 CNVs.
Recently the 15q11.2 BP1-BP2 deletion has been reviewed, emerging as a microdeletion syndrome with low penetrance and variable expressivity being the CNV frequently inherited from a healthy parent. This microdeletion is considered to be a risk factor for several neurodevelopment disorders. For the reciprocal duplication the picture has been less conclusive.
Aiming for a better understanding of the clinical significance of this CNV, we collected patients with intellectual disability and/or other clinical features, referred for microarray testing, gathering clinical details for the ones with the duplication. Data was collected from two genetic laboratories.
With a total of 1545 patients, we identified eleven carrying the duplication at 15q11.2 BP1-BP2. It was possible to assess inheritance in only four cases, all inherited from a healthy parent. All patients presented intellectual disability, and facial dysmorphism was the second most common feature observed. Microcephaly, autism, congenital abnormalities, dystonia and cataplexy

where reported individually.
The magnitude of the effect of 15q11.2 duplication remains elusive, and the outcome unclear, posing a major challenge to genetic counseling. Nevertheless, we expect the collection of more of these cases will establish this gain, as it happened with the reciprocal deletion, as a microduplication syndrome with low penetrance and variable expressivity.

P08.02

Copy number variation at chromosome 16p13.11 in Estonian patients

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There are many copy number variations (CNVs) that are associated with susceptibility for neurodevelopmental disorders. One such novel CNV is deletion and duplication at chromosome 16p13.11 whose clinical significance is becoming more ascertained.
CNVs at 16p13.11 show broad phenotypic manifestations and incomplete penetrance. They are significantly enriched in individuals affected by developmental delay/intellectual disability, autism, epilepsy, dysmorphic features and congenital anomalies. Pathogenic 16p13.11 CNVs vary in size, but harbour the critical region called interval II (chr16: 15.48-16.32 Mb, GRCh37/hg19).
We investigated the burden of CNVs at 16p13.11 (HumanCytoSNP-12 v2-1 BeadChips; Illumina Inc.) in a sample of 3,212 individuals with a range of neurodevelopmental conditions, clinically referred for chromosomal microarray analysis. Cases were compared with 14,747 controls from the Estonian Genome Center. We identified 16 patients with CNV within the 16p13.11 region, representing ~ 0.5 % of the patients analyzed, as compared to ~ 0.15 % in the Estonian general population. Eight cases were with deletion and eight with duplication in that region, including one prenatally diagnosed case.
We found that patients with CNV in 16p13.11 present with varied clinical features as previously described. These features are incompletely penetrant. All deletions and duplications identified encompass the critical region of the CNV. The sizes of the rearrangements vary between 0.3-2.7 Mb.
Our findings confirm that genomic abnormalities at chromosome 16p13.11 predispose to a range of neurocognitive and developmental disorders in individuals who carry them

P08.03

Phenotypic variability associated with the recurrent 1q21.1 copy number variant: eight new cases

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Introduction: The chromosome 1q21.1 locus is a region with a high susceptibility to recurrent deletions and duplications. These disorders have incomplete penetrance and variable expressivity. Hence the clinical significance of this copy-number variation (CNVs) can be difficult to evaluate (from development delay, mild to moderate intellectual disability, autism, psychiatric and behavioural problems to normal phenotype).
Materials and Methods: Our laboratory has examined 664 samples by targeted array comparative genomic hybridization using an (Cytochip ISCATM 8x60 v2.0 Illumina). Samples were received from patients with mental retardation, epilepsy, autism and/or congenital anomalies. When parental samples were available, the familiar study was performed.
Results: We have identified 5 patients with microdeletion and 3 patients with microduplication 1q21.1. One patient with microdeletion was referred for microcephaly and the other 4 patients for developmental abnormalities or mental retardation. In two cases the parents are also affected. We have only one case of parental samples and the deletion was of paternal origin. Two patients with microduplication were referred for epilepsy and the other one by autism. All have macrocephaly. In two cases we have the parental samples and the duplication was of maternal origin in both cases.
Conclusions: In our group of patients the recurrent 1q21.1 CNV is the most frequent anomaly (1,2 %). The macrocephaly is constant in patients with duplication 1q21.1. The family study is inherited origin in all our studied cases. The clinical variability and the incomplete penetrance make genetic counselling very difficult, especially in prenatal diagnosis.

P08.04

Identifying candidate genes for 2p15p16.1 microdeletion syndrome using clinical, genomic and functional analysis

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Introduction: The 2p15p16.1 microdeletion syndrome has a core phenotype consisting of intellectual disability, microcephaly, hypotonia, delayed growth, common craniofacial features, and digital anomalies. So far, more than 20 cases with this syndrome have been reported in literature; however, the size of the deletions and their breakpoints vary making the identification of candidate genes challenging. Recent reports pointed to four genes (XPO1, USP34, BCL11A and REL) which were included, alone or in combination, in the smallest deletions causing the syndrome.
Results: Herein, we describe 8 new cases with 2p15p16.1 deletion and review all published cases. We demonstrate functional deficits for the above 4 candidate genes using patient lymphoblast cell lines (LCLs) and knockdown of their orthologs in zebrafish. All genes were dosage sensitive based on reduced protein expression in LCLs. In addition, deletion of XPO1, a nuclear exporter, co-segregated with nuclear accumulation of one of its cargo molecules (rpS5) in patient LCLs. Other pathways associated with these genes (e.g. NF-kB and Wnt signaling as well as DNA damage response) were not impaired in patient LCLs. Knockdown of xpo1a, rel , bcl11aa and bcl11ab resulted in abnormal zebrafish embryonic development including microcephaly, dysmorphic body, hindered growth, small fins as well as structural brain abnormalities.
Conclusions: Our multifaceted analysis strongly implicates XPO1, REL, and BCL11A as candidate genes for the 2p15p16.1 microdeletion syndrome.

P08.05

Haploinsufficiency of ZNF385B and neurological manifestations in 2q31microdeletion syndrome

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Microdeletion of 2q31 involving the HOXD gene cluster is a rare syndrome. The deletion of the HOXD gene cluster is thought to result in skeletal anomalies in these patients. HOX genes encode highly conserved transcription factors that control cell fate and the regional identities along the primary body and limb axes. Several patients with this syndrome have neurological manifestations. We experienced a new patient with 2q31 microdeletion encompassing the HOXD gene cluster and some neighboring genes including the ZNF385B. The patient showed digital anomalies, growth failure, epileptic seizures, and severe intellectual disability. MRI showed delayed myelination and a low signal intensity in the bilateral basal ganglia. Abnormal MRI findings in the 2q31.1 microdeletion syndrome have been reported in some other patients. They were also haploinsufficient for ZNF385B. The ZNF385B is a zinc finger protein expressed in brain. We suggest that haploinsufficiency of ZNF385B is responsible for the neurological features of this syndrome.

P08.06

2q37.3 deletions among children with intellectual disability, autism and/or congenital malformations

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Introduction. 2q37.3 deletion syndrome is considered to be a rare chromosomal disorder associated with variable clinical features, among which are intellectual disability (ID), autistic features, brachydactyly, short stature, hypotonia and obesity. Some facial features are characteristic for the syndrome (i.e. round face, frontal bossing, arched eyebrows, deep-set eyes, upslanted palpebral fissures, epicanthal folds, thin upper lip, minor ear anomalies). Haploinsufficiency of HDAC4 seems to be the most critical for the phenotypic spectrum. Here, we have attempted to assess chromosome