levels are positive on the 1st tier. 2nd tier testing of known immunodeficiency genes using the Ion AmpliSeq 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, IL4G, RECP14 and ADA had TREC of 0, 0, 0, 1, 10, 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.

Conclusions: TREC as 1st tier, and HTS SCID gene panels 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


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 chr2q21.2 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/μl blood.

Results: All previously diagnosed patient DBS were confirmed to contain ≤5 TREC. Normal DBS ranged 46-276, average of 132 TREC. Dilution experiments showed LOQ at 14 TREC. LOD was 11 TREC. Precision experiments showed <20%CV for intra-assay (at 54 TREC) and inter-assay (at 60 TREC) 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 to sus M. D. Giannakouros1, E. V. Elisaf, T. I. Karkashidou, M. D. Giannakouros2, E. P. Charalambous, E. Radav1, R. Coreas1; 1Genomika and Genetic Diagnosis Unit, NICIUVA Biomedical Research Institute, Valdemar, Spain; 2Department of Oncology and Medical Oncology, University of Athens and Aristotelian University of Thessaloniki (CIBERDEM), Institute of Health Carlos III, Madrid, Spain.

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 (FOXO3) involved in p53 pathway were altered in SLE, and associated with immune activation.

Materials 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 reverse transcription PCR (qRT-PCR) in the urinary sample.

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 intermedia 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 (-1480C>A) detected for the first time in the Greek population (β-thalassaemia heterozygote, HbF 19.5%) in coexistence with one published variation of unknown significance (c.544T>C;p.Chr182Leu). Two alternations, not yet recorded (g.1980C>A, c.3813A>C), were found in β-thalassaemia heterozygotes, (HbF: 5.9% and 10.1%) and a frequent polymorphism (c.3047T>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 HbF regulation.

P08 Intellectual Disability

P08.01
The critical 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 syndromic/ non syndromic variable number copies (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 catacteply
POI02
Co py number variation at chromosome 16p13.11 in Estonian patients
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Introduction: The 1p36.1 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 patient LCLs. In addition, deletion of XPO1, a nuclear exporter, co-segregated with nuclear accumulation of one of its cargo molecules (prSS) 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 xpo1, rel, bcl11a and bcl11b 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.

POI03
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 developmental 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 ISCMAT 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 paternal 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.