racteristics of Marfan syndrome and lately FBN1 polymorphisms drew more attention to cause sporadic DPATA. Based on LeMaire et al study in 2011, we investigated polymorphisms of the FBN1 gene (rs2118181, rs1036477, rs10519177, rs755251, rs4774517) in a case-controlled study for DPATA from Lithuanians.

We studied 312 patients who had undergone aortic reconstructive surgery for DPATA. Patients were subdivided into test groups according to the DPA-TA phenotypes: ascending aortic aneurysms, dissectionsandpost-stenotic-dilatation. The control group (n=472) was obtained from a random sample screened within epidemiological studies of the Lithuanian population. The FBN1 polymorphisms were investigated by real-time polymerase-chain-reaction amplification. Fisher's exact test, $\chi 2$ test and allelic association odds ratio were used for statistics. The distribution of genotypes was conformable with Hardy-Weinberg equilibrium ($p>0.15$).

We observed that minor alleles of all five FBN1 SNPs were significantly associated with aortic dissection with OR 2.13-2.59, $p<0.001$, and two SNPs: rs2118181 and rs1036477- with an increased risk of ascending aortic aneurysm with OR 1.67, CI 95% 1.61-2.40.There were no significant associations between all studied FBN1 SNPs and post-stenotic aortic dilatation.

Minor alleles of all SNPs investigated might be considered as risk alleles for aortic dissection and two of them (rs2118181, rs1036477) are increasing odds for aortic aneurysm formation.

PM05.48

Upregulation of Antigen Presentation Pathway under the treatment with neuropropeptide Semax in a rat model of brain focal ischemia

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Neuroprotective synthetic oligopeptide Semax composed of a fragment of ACTH4-7 and C-terminal tripeptide Pro-Gly-Pro is used for therapy of acute stroke. The molecular mechanisms of its neuroprotective action have been hitherto unknown. The response of the transcriptome of ischemized rat brain cortex tissues to the action of Semax in the male Wistar rat brains was investigated. The intraperitoneal injection of peptide was done at 15 min, 1, 4 and 8h after permanent middle cerebral artery occlusion (pMCAO). mRNA expression change was analyzed in 24h groups versus 3h groups following pMCAO and "pMCAO + Semax". The Illumina RatRef-12 Expression BeadChip was used in our study. The action of Semax enhanced the expression of 17 genes (Ap1, B2m, Cd74, Hla-C, Hla-Dma, Hla-Dmb, Hla-Dqa1, Hla-Dra, Hla-Drb1, Hla-E, Psmb8, Psmb9, Tapbp) belonged to the major histocompatibility complex (MHC). We identified significant overlap between 13 of these genes and the Antigen Presentation Pathway ($p=4.4E-11$). BioRank Ingenuity ranking system assigns to this pathway the high scores that means that this set of changed expression genes hit a critical component of the pathway in the Ingenuity Canonical Signaling Pathway library. Besides we observed changed levels of transcripts of 7 genes (Cd74, Hla-A, Hla-Dra, Psmb8, Psmb9, Tap1, Tapbp) belonged to the MHC in the "ischemia" group during one day after pMCAO. But for the Antigen Presentation Pathway this set of genes was scored lower than in the case of neuropeptide treatment. Our data assume that Semax affects the immune response during the active stage of ischemia.

PS05.47

A human laterality disorder associated with a homozygous WDR16 deletion

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Visceral asymmetry is determined through embryonic ciliary motion. A failure to generate the normal left-right (L-R) asymmetry during early stages of embryogenesis may result in severe anatomical abnormalities, including heterotaxy syndrome (HS) which consists of abnormal L-R axis arrangement of the abdominal and thoracic viscera and situs inversus totalis (SIT) which manifests by mirror image asymmetry of the internal viscera. HS is at times accompanied by complex congenital cardiovascular anomalies whereas SIT is frequently associated with Primary Ciliary Dyskinesia (PCD). The genetic etiology of defects not associated with PCD is largely unknown.

In this study we investigated the cause of situs anomalies, including HS and SIT (PCD excluded), in a consanguineous family.

Whole exome analysis including thorough coverage analysis, revealed a homozygous deleterious deletion in the WDR16 gene - chr17. hg19:g.9481617_9489649del8033.

The finding was confirmed both by cDNA analysis and by the results of Multiplex Ligation-dependent Probe Amplification analysis which confirmed segregation of the deletion in the family. Serial PCR reactions using intronic primer sets, resulted in the amplification of a genomic fragment which contained the breakpoint.

WDR16 protein was previously proposed to play a role in cilia-related signal transduction processes: the rat Wdr16 protein was shown to be confined to cilia possessing tissues and severe hydrocephalus was observed in the wdr16 gene knockdown zebrafish.

The phenotype associated with the homozygous deletion in our patients suggests a role for WDR16 in human laterality patterning. Exome analysis is a valuable tool for molecular investigation even in cases of large deletions.

PS05.49

Hereditary hemorrhagic telangiectasia (Rendu-Osler-Weber syndrome): first results of molecular genetic testing in the Czech republic

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Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant disorder characterized by an aberrant vascular development. HHT affects approximately 1 in 5000 people and is caused by pathogenic variants in a number of genes involved in the TGF-β signaling pathway. Endoglin (ENG) and activin receptor-like kinase-1 (ALK1/ACVRL1) encode proteins expressed on vascular endothelial cells. These genes are casually related to HHT. The clinical diagnosis of HHT is based on recurrent epistaxis due to telangiectases and arteriovenous malformations (AVMs) which can occur in the pulmonary, cerebral and hepatic circulation leading to stroke, internal hemorrhage, and severe anaemia. We used classical sequencing approaches to perform molecular characterization in 18 clinically affected unrelated probands with the suspected diagnosis of HHT, and detected a total of 7 different mutations in the two genes. Two mutations were identified in the ENG gene, both deletions, one of which was novel, in exon 8 and exon 11. There were tested also several family members of these probands and we identified 5 and 2 mutation carriers, respectively. Three of five mutations identified in the ALK1/ACVRL1 gene were novel and comprised missense mutation in exon 6, deletion in exon 3 and small indel in exon 8. No mutations were found in ENG/ACVRL1 in 11 probands. Genetic testing can confirm the clinical diagnosis in individuals and identify presymptomatic mutation carriers. Once a famililal mutation is identified, relatives at risk can be tested. Individuals with a mutation are identified for intensive clinical surveillance. We offer genetic counseling for at-risk relatives.

PM05.50

Sarcomeric gene mutations in Turkish families with hypertrophic cardiomyopathy

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Background: There is little knowledge about familial hypertrophic cardiomyopathy in Turkey. In this study, our aim was to determine a causing mutation in three sarcomeric genes (MYH7, MYBPC3 and TNNT2) in Turkish families with HCM and high-risk for sudden cardiac death (SCD).

Materials and methods: The study included twelve index cases of early onset (<40 years) clinically diagnosed HCM patients with a positive family history for HCM and SCD. All participants were evaluated with a detailed history, physical examination, 12-lead electrocardiography and two-dimensional echocardiography. DNA was extracted from peripheral blood and coding regions and flanking intronic sequences of MYH7, MYBPC3 and TNNT2 genes were screened using array-based re-sequencing. All novel variants and known mutations were confirmed with Sanger sequencing. After the causal mutation in family members was screened.

Results: From 12 index cases, a known missense mutation was found in 6 individuals and also novel missense mutation was found in 2 individuals. Four different causal mutations in the cardiac-beta myosin heavy chain (MYH7) gene, one in the cardiac myosin binding protein C (MYBPC3) gene and one in the cardiac troponin T (TNNT2) were found. In addition, two novel intronic variants in MYH7 and MYBPC3 genes were found in 2 different index cases. Detection of the novel missense mutations and intronic variants within the family members and control population is ongoing.

Conclusion: Our preliminary result provides a general view of the familial

hypertrophic cardiomyopathy with high-risk for SCD and highlights the importance of mutation screening for these three genes in Turkey.

PS05.51

Disease causing variation is differentially distributed in MYH7 but not in MYBPC3 in patients with hypertrophic cardiomyopathy

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Hypertrophic cardiomyopathy (HCM) is an inherited disorder of the heart muscle. HCM has been linked to dominant genetic variants in sarcomere genes, most commonly in *MYH7* and *MYBPC3*. The *MYH7* and *MYBPC3* gene products interact but have distinctly different functions: *MYH7* encodes the myosin head, the molecular motor that binds and pulls actin, while *MYBPC3* is a regulatory protein that binds to both the myosin and actin. Despite these functional differences, variants in both genes lead to a similar presentation of HCM. To investigate the differences between the variant profiles of *MYH7* and *MYBPC3*, we combine published and unpublished data from three centers (Stanford University, the Mayo Clinic, Harvard University). The combined dataset included results from 4349 patients. In *MYH7*, 215 out of 234 (91.9%) unique damaging variants are missense, while only 164 out of 343 (47.8%) are missense in *MYBPC3* (Fisher $p=1.25x10^{-6}$). We compared variant locations in *MYH7* and *MYBPC3* from HCM patients with those from 60,706 individuals in the Exome Aggregation Consortium (ExAC). We find a significant difference in the distribution of HCM and ExAC variant locations in *MYH7* (KS $p=3.95x10^{-13}$), but not in *MYBPC3* (KS p=0.462). The peak of differential variant density in *MYH7* covers the head, the lever arm, and the beginning of the tail of the myosin heavy chain molecule. Our results suggest differing regional biophysical contributions to the pathogenicity of disease for *MYH7* and *MYBPC3*.

PM05.52

Cardiac Ankyrin Repeat Protein (CARP/Ankrd1) gene variants in Hypertrophic Cardiomyopathy

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CARP/Ankrd1 belongs to the muscle ankyrin repeat protein (MARP) family involved in a mechano-signaling pathway that links myofibrillar stress response to muscle gene expression. Also, CARP/Ankrd1 has an important role in transcriptional regulation, myofibrillar assembly, cardiogenesis and myogenesis. Few studies supported a role of CARP gene variants in the etiology of hypertrophic (HCM) and dilated cardiomyopathy. We have performed screening for mutations/variants in Ankrd1 coding sequences in 50 familiar or idiopathic HCM patients. Two missense heterozygous CARP variants in exon 2 (P52A and R66Q) were identified, each in one patient. Preliminary functional analysis of these variants on protein level were performed in rat neonatal cardiomyocytes by Fluorescent Recovery After Photobleaching (FRAP) assay. The results of the FRAP experiments showed difference in mobility between wt and P52A variant, while R66Q exerted similar behavior as wt protein. Our findings point to possible disruption of the protein-protein interaction and disturbance of the normal cardiac signaling in cardiomyocytes caused by P52A allele variant of CARP/Ankrd1.

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PS05.53

Investigation of Pathogenic Genes in Chinese Hypertrophic Cardiomyopathy Patients by Whole Exome Sequencing

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Hypertrophic cardiomyopathy (HCM) is a cardiovascular disease with high heterogeneity. The limited knowledge about ~40% patients suggests that it needs to be investigated to understand the pathogenesis of the disease. A large number of variants were identified by whole exome sequencing in seventy-four HCM patients passing Sanger method sequencing eight HCM causative genes. After filtering against multiple databases and functional filter, 3228 SNPs and 475 InDels in 3046 genes were identified. TADA model was then applied using exome sequencing data of 2000 controls and 74 cases and 99 genes gained priority, with DNAH11, OBSCN ranking the first and second place, respectively. Associated analytical tools as DAVID and IntPath were also adopted to explore the novel mechanism underlying the HCM phenotype. The results showed that various genes and gene sets related to cytoskeleton, ATPase activity, dynein and calcium transport played critical roles in the pathogenesis of HCM, with a lot of other processes as well, implicating many processes involved in the HCM phenotype. We also found two novel OBSCN variants that co-segregated with the HCM patients in two HCM families. Additionally, we described the OBSCN role in HCM. Our study provides a way for exploring the pathogenesis of a less understood HCM cohort.

PM05.54

Mutational spectrum of Filamin C (FLNC) in hypertrophic cardiomyopathy

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Exome sequencing identified the Filamin C (*FLNC*) as a hypertrophic cardiomyopathy (HCM) candidate gene.[1] The *FLNC* putative mutations segregated with the disease in several families. We also demonstrated that patients with *FLNC* mutations showed marked sarcomeric abnormalities in cardiac muscle, and functional studies revealed that these *FLNC* variants resulted in the formation of large filamin C aggregates. Our aim was to characterize the mutational spectrum of *FLNC* in a large cohort of HCM patients. We performed a Next-generation sequencing of *FLNC* in 335 HCM patients, including 117 sarcomeric-mutation carriers. We identified a total of 25 HCM index cases who were heterozygous carriers of putative mutations, 21 in the 218 patients without sarcomere-mutation (10%) and 4 in the 117 sarcomere-positive cases (3%; $p<0.05$). Only five of the 25 *FLNC* variants were reported in healthy subjects in the Exome Sequencing Project (ESP) database, but all of them at a frequency <0.001%. Three variants, p.A1247V, p.A1539T, and p.A2340V, were found in two index cases. In nine of the 14 families we had several affected members, and all were mutation carriers. Interestingly, some of the *FLNC* mutations were linked to sudden cardiac death episodes. In conclusion, our work suggested that *FLNC* mutations were a significant cause of HCM.

[1]Valdés-Mas R *et al.* Mutationsin filamin C cause a new form of familial hypertrophic cardiomyopathy. Nature communications. 2014;5:5326).

PS05.55

The Clinical Utility of a Proton-Ion Ampliseq-based Cardiac Gene Panel

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Introduction: Whole exome sequencing (WES) and whole genome sequencing (WGS) are increasingly being used in clinical practice. However, these approaches continue to be expensive, time consuming and difficult to interpret. So far, clinical WES studies have yielded diagnostic results in ~ 25% of cases. Hence, a complementary easier, equally sensitive method needs to be developed.

Method: A 406 cardiac related (cardiomyopathy, congenital heart disease, arrhythmias, vascular aneurysms) gene panel (with 10490 amplicons) based on Ion Torrent AmpliSeq technology was developed. So far, 243 gDNA samples were sequenced using the Proton-Ion sequencing instrument.

Results: In total, 4.13 Gb were mapped with 94% on target and base reads with 370 reads per amplicon and overall average base coverage depth of 325. In the 243 samples, a total of 51 variants (including 2 homozygous CACNA1C and SCN5A homozygous mutations) with an overall clinical sensitivity of 51/242 (21%) was found. Sub-panel diagnosis based clinical sensitivity was 32% for cardiomyopathy, 10% for congenital heart disease, 31% for arrhythmias and 29% for aortic aneurysms/Marfan syndrome.

Conclusion: This custom made, comprehensive cardiac gene panel appears to have a clinical sensitivity comparable to that of the current WES with the advantage of faster turnaround time and far fewer variants to analyse. Only in 6% of the genes on this panel were relevant DNA sequence variants identified. Hence, a tiered approach starting with a custom gene panel followed by WES is probably a more efficient clinical approach.

PM05.56

Homozygous, and compound heterozygous mutation in 3 Turkish family with Jervell and Lange-Nielsen Syndrome

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