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Abstracts

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of the myocardium. Conventional genetic testing of genes encoding desmosomal components results in a diagnostic yield of about 50% of ACM probands.
Aim - To search for large deletions/duplications in desmosomal genes in a large cohort of ACM index cases.
Methods and Results - Genetic screening for large deletions/duplications in 5 desmosomal genes was carried out in 68 unrelated index patients diagnosed affected with ACM according to revised 2010 Task Force criteria, and resulting negative for pathogenic point mutations in the same genes. Quantitative real-time PCR (qPCR) experiments were performed on an ABI PRISM 7900HT Sequence Detector by using at least three different sets of primer pairs located within exons from the proximal to the distal part of each gene. In 3 patients (4.4%) we identified a plakophilin-2 (PKP2) copy number reduction when compared to control samples thus suggesting a large heterozygous gene deletion. The PKP2 gene deletion was confirmed by Multiplex ligation-dependent probe amplification with the SALSA MLPA kit P168 (MRC-Holland). Haplotype analysis revealed a conserved haplotype among the PKP2 mutation carriers, strongly indicating a common founder.
Conclusions - Among desmosomal genes, only PKP2 shows large deletions of the entire coding region. These findings support the importance of expanding genetic testing in ACM patients with inclusion of PKP2 large deletion analysis when the results of conventional sequencing are negative.

PM05.10
A novel locus on chromosome 19p13.3 linked to arrhythmogenic cardiomyopathy

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BACKGROUND:
Arrhythmogenic cardiomyopathy (ACM) is an autosomal dominant myocardial disorder at risk of sudden death in the young and athletes. Thirteen causative genes have been found, with a central role of the desmosomal genes. Since causative mutations in ACM genes have been detected in about 50% of probands, additional disease genes remain to be identified.
METHODS and RESULTS:
In a large ACM family, where the proband resulted negative for mutation screening of desmosomal genes, genome-wide linkage analysis highlighted a shared region of 2 Mb on chromosome 19p13.3 (multipoint LOD score=3.85). CNV analysis was carried out in order to exclude the presence of structural variations. Whole exome sequencing was performed in 4 affected patients, through two different platforms (HiSeq2000 Illumina, Ion Torrent) at a mean coverage of 80X. Sequencing data didn't reveal the presence of any novel variant shared by the 4 subjects, neither into the linkage region nor in the rest of the exome. Exons with insufficient reads (≤15 depth) of the 13 ACM genes and of the genes inside the critical region were further evaluated by Sanger sequencing but no additional coding mutations were found. Only a novel intronic variant (c.766+8C>A) in TMEM43 gene was identified. The segregation of this variant among all the available family members excludes an association with the disease phenotype.
CONCLUSION:
In this ACM family showing no mutations in known ACM genes segregating with the disease phenotype, a novel locus was mapped on chromosome 19p13.3 and a critical region of 2 Mb was defined.

PS05.11
Inducible pluripotent stem cell technology as a tool to study Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) in a Maritime family

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ARVC is an inheritable disease featuring potentially lethal arrhymias and heart failure characterized pathologically by fibrofatty replacement of cardiaomyocytes (CMs). We have ascertained a large Maritime family segregating a form of ARVC that is mutation-negative for all genes currently known to cause ARVC, suggesting that a novel variant must be responsible. Using DNA derived from formalin fixed, paraffin embedded tissue, as well as from blood or saliva of known carriers and relatives, we have performed extensive genetic investigation to identify the novel mutation responsible for the disease in this family (i.e. SNP genotyping, haplotype analysis, whole-exome and whole-genome sequencing). We have identified candidate genomic regions shared among affected individuals, however the precise causative mutation remains elusive. To assist in identifying the causative factor and to study the underlying disease mechanisms, we have used a novel inducible pluripotent stem cell (iPSC) technique to generate disease specific CMs that were derived from B-lymphocytes of ARVC patients. Human iPSC lines were successfully developed by using episomal plasmid vectors to over express reprogramming factors. CMs were then differentiated from iPSC by modulating Wnt/β-catenin signaling. Currently, gene expression analyses with RNAseq from RNA isolated from iPSC derived CMs that were generated from affected and healthy control samples are underway. The iPSC technique used in this project may help identify the specific mutation that causes this specific form of ARVC, enabling life-saving intervention in at-risk individuals. Additionally, this technique offers a potential pathway for identifying causal variation in diseases in which coding-sequence changes are not obvious.

PM05.12
Bioinformatics analysis of the genomic candidate markers for diagnostic panel of atherosclerosis

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Despite the significant achievements in the field of medicine and biology, morbidity and mortality from atherosclerotic lesions remain at a high level, and the range of adequate antiatherosclerotic therapy goals is still limited due to the lack of validated biomarkers. Therefore, the main goal of our research is the development of a new reliable genomic indicators panel for early, pre-clinical diagnostics of possible future disease.
We performed a bioinformatics analysis of potential candidate genes directly or indirectly involved in the pathogenesis of atherosclerosis. The analyzed genes list included lipid metabolism, matrix metalloproteinases, folate cycle, oxidative stress genes and some others. The motifs search was implemented using bioinformatics package MEME Suite.
In the investigated genes vicinity we detected 670 motifs, homologous pre-mi-RNA, and 4300 motifs, homologous mature mi-RNA. The average mi-RNA distribution density in genomic sites ranged from 0 to 2.2 pre-mi-RNA per 1000 p.n. and mature mi-RNA - from 0 to 6.9 per 1000 p.n. Inside the investigated genes we identified 433 motifs, homologous pre-mi-RNA, and 2780 motifs, homologous mature mi-RNA. The average motifs distribution density amounted to 0.4 per 1000 p.n. for pre-mi-RNA and 3.3 per 1000 p.n. for mature mi-RNA. The most frequently encountered were motifs, homologous mmu-mir-466i, hsa-mir-5096, hsa-mir-1273g, ppy-mir-1268 and hsa-mir-619. According to the MirTarBase data we established that hsa-miR-138-5p is the multigene regulator for the studied genes groups.
The obtained results could be used to develop atherosclerosis diagnostic panel.
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PS05.13
Whole exome sequencing identifies a novel germline mutation in calcium ion channel; associated with atrial fibrillation.

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Introduction - AF is the most prevalent sustained cardiac arrhythmia, responsible for considerable morbidity and mortality. Due to the complex pathophysiology of AF the underlying mechanisms are incompletely understood. In the few cases of familiar form of AF, where the causative mutation has been found, it has been identified in genes encoding ion-channels involved in sodium or potassium handling. Nevertheless in the majority of cases with familiar forms of AF the cause is still unknown.
Methods and Results - Whole exome sequencing was performed on seven individuals from a Danish family, presumably with a highly penetrable monogenetic form of AF. Quality control showed that the whole exome could be investigated sufficiently.
Analysis, following Broad institute current best practices, revealed 2 novel variants (not found in over 60,000 controls, of which 2000 Danish). Interestingly one of the novel mutations was found in a calcium ion channel. In silico predictions supported the pathogenic potential of this variant, predicted damaging in 7/8 prediction tools.
Discussion - In this study a calcium ion channel variant that co-segregated