Abstract: P508

Massively parallel sequencing of patients affected with arrhythmogenic cardiomyopathy by a targeted gene panel identified a novel nonsense mutation in TP63 gene.

Authors:
G Poloni1, M Calore1, A Lorenzon1, G Thiene2, C Basso2, D Corrado2, B Bauce2, A Rampazzo1, M De Bortoli1, 1University of Padova, Department of Biology - Padua - Italy, 2University of Padova, Department of Cardiac, Thoracic and Vascular Sciences - Padua - Italy,

Topic(s):
Basic Science - Cardiac Diseases: Cardiomyopathies

Citation:
Cardiovascular Research (2018) 114 (Supplement 1), S123

Funding Acknowledgements:
TRANSAC Strategic Research Grant CPDA133979/13, University of Padua, Italy; The University of Padua Research Project (PRAT) CPDA133979

BACKGROUND: Arrhythmogenic Cardiomyopathy (ACM) is a clinically and genetically heterogeneous heart muscle disease and a leading cause of sudden cardiac death in the young and athletes. The majority of mutations identified in ACM patients involved genes encoding proteins of the intercalated disc but still 40% of patients remained genetically unidentifed.

PURPOSE: The aims of this study was: 1) the identification of the genetic cause in 40 ACM patients; 2) the identification of putative novel genes associated with ACM.

METHODS: DNA samples from 40 ACM probands negative for mutations in the 3 major ACM genes (DSP, PKP2 and DSG2) were screened by using a targeted gene panel consisting of 67 genes, 14 known ACM genes and 53 candidate genes. For each sample the exonic and intronic flanking regions of the investigated genes were enriched and sequenced using 150bp paired-end reads on a massively parallel sequencing platform. Only the variants covered at least 15X have been considered as reliable variants. Taking into account that the allele frequency of ACM in the general population span from 0.01% and 0.025%, a genetic variant has been considered a ‘mutation’ if its minor allele frequency (MAF) is =0.01%. In silico predictions of pathogenicity of rare nonsynonymous variants was performed with 3 different algorithms. All the mutations were validated by direct Sanger sequencing.

RESULTS: About 1,950,000 reads per sample covering the coding and flanking intronic regions of 67 genes have been produced. The sequencing depth resulted in a median coverage of 267X (ranging from 165X to 351X), with 96.45% covered at =10X. Considering only the variants covered at least 15X, on average, 445 variants per sample have been detected, 409 were SNPs and 36 InDels. Discarding synonymous single-nucleotide variants and excluding TTN gene, we identified a total of 25 exonic mutations (2 nonsense and 23 missense) and 2 splice-site variants. About half of patients resulted to carry a mutation, 12 (30%) showed a single mutation and 6 (15%) showed multiple mutations. In 9 out of 40 probands, one or more mutations have been found in genes associated with ACM and/or with other inherited heart diseases. Among missense and nonsense mutations in candidate genes identified in 9 (22.5%) patients, a nonsense mutation c.796C>T, p. (R266*) was detected in a strong ACM candidate gene encoding for p63, a DNA binding transcriptional factor, involved in cardiomyocyte differentiation and in Wnt signalling regulation. The same patient carried also a novel missense variant in TLN1 gene (c.3707A>G, p.Q1236R), whereas the affected mother resulted to carry only the TP63 mutation.

CONCLUSIONS: This targeted gene panel resulted to be a valid approach for both diagnostic and research
Massively parallel sequencing of patients affected with arrhythmogenic cardiomyopathy by a targeted gene panel identified a novel nonsense mutation in TP63 gene.

P508

purposes in patients affected with ACM. Our approach led to the identification of the genetic cause in 9 ACM patients and to the identification of TP63 as a possible disease gene.