INTERPRETATION OF NON-CODING VARIANTS IN INHERITED CARDIOMYOPATHIES ASSOCIATED TO SUDDEN DEATH

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Cardiomyopathies are inherited conditions characterized by structural or electrical alterations of myocardium, and represent the most common causes of sudden cardiac death (SCD) among young people. They are autosomal dominant diseases, with variable penetrance and expressivity. When a definitive mutation is found in the proband it is possible to search the mutation in relatives, identifying the asymptomatic subjects at risk of developing the disease. The most common variations found in cardiomyopathy-related genes are point mutations, which can be classified as pathogenic when cause the introduction of a stop codon, affect the canonic splicing site or if they are functionally characterized. The utilization of bioinformatics tools is useful to predict the pathogenicity of mutations, but they may be doubtful to analyze the effect of point mutations located in non-coding regions [Wallis Y ACGS /VGKL 2013].
We functionally tested 5 intronic variants (MYBPC3-c.506-2 A>C, MYBPC3-c.906-7C>T, MYBPC3-c.2308+3 G>C, SCN5A-c.393-5 C>A, ACTC1-c.617-7 T>C), found in 5 patients affected by inherited cardiomyopathies (hypertrophic cardiomyopathy or Brugada syndrome), related to SCD. The MYBPC3-c.506-2 A>C mutation was analyzed in mRNA extracted from peripheral blood of the patient. The analysis revealed the loss of the canonical splice site and the utilization of an alternative splicing site, causing the loss of first 7 nucleotides of the exon 5 (MYBPC3-G169AfsX14). When patients mRNA was not available, we generated minigene constructs, transfected in HEK-293 cells. The minigene assay showed the MYBPC3-c.2308+3 G>C and SCN5A-c.393-5 C>A produced an altered pre-mRNA processing, resulted in the skipping of the involved exons. On the contrary no alterations were found in ACTC1-c.617-7 T>C minigene. In addition, we are performing in vitro analysis of MYBPC3-c.906-7C>T variation.
In conclusion, the in vitro analysis of these mutations shows different splicing alterations consistent with the diagnosis, except for the ACTC1 variation. The identification of causative mutations is an integral part of the diagnostic process. Thus, the evaluation of pathogenic effects by in vitro analysis can be helpful for interpretation of non-coding variants and to make a correct molecular diagnosis.