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ABSTRACT OF PhD THESIS

Genetic Aspects in Congenital Heart Defects

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TÂRGU MUREȘ 2023

Introduction. Congenital heart diseases (CHD) are the most common congenital anomalies identified at birth, with an incidence of 1-1.2% among newborns. Etiopathogenesis of MCC is mainly determined by the interaction between genetic and environmental factors. The highest incidence in the CHD group is represented by sporadic and nonsyndromic forms, being most frequently associated with variants of *GATA4, GATA6, NKX2-5, TBX5, GJA1,* and *SMAD2* gene. Functional studies confirm the major role of these genes in cellular communication pathways and the regulation of gene expression in the cardiogenesis process.

Objectives. The objectives of this study consist of complex molecular analysis of patients with CHD, meaning septal heart defects and conotruncal defects in nonsyndromic forms, with the phased investigation of some genes involved directly and indirectly in the cardiogenesis process. Thus, in the first stage, we proposed to analyze the copy number variants (CNVs) for *GATA4* and *GATA3* genes, the second objective was represented by the analysis of some variants of the *AXIN1, AXIN2, TCF21, GATA4,* and *GJA1* genes. Another objective was represented by the complex molecular analysis of these patients, using next generation sequencing technique (NGS), in order to identify genetic variants associated with this pathology.

Study 1. Molecular analysis of CHD using the MLPA technique

In this study, we included 82 patients diagnosed with atrial septal defects, ventricular septal defects, and Tetralogy of Fallot in which we performed the analysis of CNVs of *GATA3* and *GATA4* genes. Using the MLPA technique and SALSA P234-MLPA kit (MRC-Holland, Amsterdam, Netherlands) we identified in 2 patients a modified signal at the level of exon 4 and exon 5 at the level of the *GATA3* gene. The results were normal for the remaining patients, with no CNVs identified at the level of the *GATA3* and *GATA4* genes.

Study 2. Molecular analysis of ~~gene~~ variants of *AXIN1* and *AXIN2* genes and susceptibility for CHD

In this study, we genotyped 214 subjects, namely 111 healthy children and 103 patients diagnosed with ASD(51.5%), VSD(12%), TF(15%), DORV(3%), ASD and VSD(18.5%). The genotyping of the rs370681 *AXIN1* and rs2240308 *AXIN2* polymorphisms was performed by using the Real-Time PCR 7500 Fast Dx system and for the rs1805105 and rs12921862 polymorphisms of the *AXIN1* gene we used the Restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) technique. Statistically significant differences between the control and patients group were observed for rs370681 *AXIN1* and rs2240308 *AXIN2.* The rs1805105 and rs12921862 of *AXIN1* gene variants analysis identified an insignificant difference between these two groups. Haplotype analysis for investigated polymorphisms of the *AXIN1* gene (rs12921862 rs370681 rs1805105) revealed an increased risk of CHD in the presence of C-C-C and C-C-T haplotypes.

Study 3. rs12190287, rs804280, rs73203482 and rs2071166 polymorphisms analysis of *TCF21*, *GATA4* and *GJA1* gene and susceptibility to CHD

In this study, we analyze the polymorphisms rs12190287 *TCF21*, rs804280, rs73203482 *GATA4*, rs2071166 *GJA1,* and susceptibility of CHD. We included 196 pediatric patients of which 111 represent the control group and 85 were included in the patients' group. Distribution of homozygous wild-type genotype AA was 97.5% in the patients' group, respectively 98% in the control group for the rs2071166 polymorphism, the frequency being similar to that reported in the European population. The rs12190287, rs804280, and genotype analysis revealed no significant differences between the variant genotypes of the investigated polymorphisms and CHD. Also, the allele distribution analysis of variants at the *TCF21* and *GATA4* genes also did not identify a statistically significant association.

Study 4. Molecular analysis of CHD using next generation sequencing techniques

The NGS analysis was performed using a custom panel in which 16 genes were included and the study group consisted of 40 patients with different forms of MCC. The results analysis, using the ClinVar database, revealed a pathogenic deletion namely TBX5(c.593del), and another four variants were identified for *GATA4, MYH6, and SRCAP* genes, reported as uncertain significance. The functional impact of data analysis identified 13 variants that interested 6 genes (*SRCAP, TBX5, MYH6, MYH7, NKX2-5, NOTCH3*). In 55% of cases, included in this study, c.5653delT of the *SRCAP* gene was identified, with a possible pathogenic effect according to the American College of Medical Genetics classification (PVS1, PM2).

Conclusions (general)

The analysis of the CNVs of the *GATA3* and *GATA4* genes did not reveal the presence of deletions or duplications for the investigated patients group, but the polymorphisms analysis of *AXIN1* gene variants identified an increased risk for CHD in the presence of C-C-C or C-C-T haplotype of rs12921862, rs370681, and rs1805105 polymorphisms. The variant allele for the rs2240308 *AXIN2* gene may also be a risk factor for the occurrence of CHD. NGS analysis identified different gene variants in a large percentage of cases (95%), and the c.5653delT of the *SRCAP* gene was identified in 55% of cases, and this may represent a gene abnormality characteristic of patients with different types of CHD**.**