Thesis: **Multiple genetic screening in the newborn with a congenital heart defect**

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## Abstract

Congenital heart defects are the most common malformation pathology present in the neonatal period. Identifying the etiology is the main step in reducing the frequency of congenital heart defects. Genetic studies are expected to provide important information about the involvement of gene mutations in congenital heart defects etiology. Accurate identification of the genetic condition that could have caused the heart defect is often challenging. Comprehension of the normal cardiovascular system development at the molecular level allowed the identification of numerous genes involved in cardiac morphogenesis. Thus, the most common occurrence mechanism is due to specific mutations in key genes, which in correlation with individual susceptibility determine the manifestation of the structural defect. The existence of several mutations within the same individual and their interaction with environmental factors increase the probability that the person will associate a congenital heart defect.

The thesis' degree of originality consists in the genetic diagnosis of newborns with congenital heart defects through the use of state-of-the-art technologies. The study was a prospective cohort, which included newborns diagnosed with isolated congenital heart defects hospitalized in a neonatal intensive care unit. Patients with a minor structural heart defect who did not require immediate surgical, interventional, or drug therapy were not enrolled. This thesis is based on three distinct studies that aimed to achieve a clinical and genetic profile of the newborn with an isolated structural heart defect.

**The first study** - The profile of the newborn with a nonsyndromic congenital heart defect. The main aim of this study was the achievement of a clinical profile specific to the newborn with a congenital heart defect, as well as to establish maternal risk factors, and prove the relationship between involved factors and the presence of the heart defect. Accordingly, a series of specific maternal, fetal, and neonatal factors were monitored. In order to meet the objectives, an individual study sheet was developed for each enrolled newborn. The study sheet included information on pre-existing maternal pathology before the pregnancy, data regarding the current pregnancy, as well as clinical and paraclinical data for the newborn. In carrying out this research, the intent was to depict the characteristic phenotype of the newborn that requires specific therapy during the neonatal period due to the association of a congenital heart defect.

**The second study** - Genetic screening of the newborn with a congenital heart defect by Multiplex ligation-dependent probe amplification. MLPA represents the standard diagnosis method in genetic disorders caused by the presence of DNA structural changes. The technique is based on multiplex polymerase chain reaction (PCR) and allows the investigation of copy-number variations (CNV), allowing the analysis of up to 60 different genomic sequences from the studied DNA. The main objective of this study was to demonstrate the involvement of CNVs in the etiology of isolated, non-syndromic heart defects. The chapter also addressed whether the MLPA technique could be used as a first-tier technique in identifying CNVs in newborns with nonsyndromic congenital heart defects. Applying the same molecular biology technique, the study tried to demonstrate the association between the 22q11.21 chromosomal region deletion and the occurrence of conotruncal heart defects in the absence of specific phenotypic features. Early identification of the genetic

origin in heart defects is important because these children are at an increased risk for postoperative complications.

MLPA testing was carried out using the SALSA MLPA P311 Congenital Heart Disease (MRC-Holland, Amsterdam, The Netherlands) kit according to the manufacturer's protocol, and a 3500xL Dx Genetic Analyzer (Thermo Fisher Scientific, USA) was used for capillary electrophoresis. Samples displaying positive results for the 22q11.2 deletion were subsequently tested with the SALPA MLPA P250 DiGeorge kit to confirm the patients' diagnosis.

**The third study** - The contribution of sequencing techniques in elucidating the etiology of isolated heart defects. Starting from the hypothesis of mutations within structural proteins, the genetic etiology of the heart defect can be identified by the use of sequencing techniques. Compared to less laborious techniques, sequencing allows an accurate analysis of most genomic variations, with a higher throughput compared to standard techniques. Variant interpretation is often challenging, especially in the case of sporadic cases, where there are limited genotype-phenotype correlations and the phenomenon of incomplete penetration occurs. The main objective of the present study was to demonstrate the involvement of sequence variants in the etiology of isolated heart defects. Another goal was to demonstrate the usefulness of Sanger sequencing as a complementary method to the standard techniques in identifying CNVs in newborns with nonsyndromic congenital heart defects. The identification of a possible causal relationship between sequence variants and the occurrence of specific structural heart defects was also addressed in this study. In order to verify the signals of apparent duplication, evidenced in the previous study, the nucleotide sequence tested by the MLPA method was included in the region of interest for Sanger sequencing. Therefore, in-house PCR primers specific for the region of interest, namely *GATA4* exon 1 as well as part of the intron, plus the exon-intron junction were used for Sanger DNA sequencing. The technique was performed with the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, USA), and amplicons were subsequently purified with the BigDye XTerminator Purification Kit (Thermo Fisher Scientific, USA), according to the manufacturer's protocol. Capillary electrophoresis was run on a 24-capillary 3500xL Dx Genetic Analyzer and the results were compared to available data from gene banks. The identification of rs35813172, rs61277615, and rs73203482 sequence variants demonstrates that, under certain conditions, they can be potentially pathogenic.

Despite knowing the specificity of each gene variant, the prevalence of these variants in the healthy population is still unknown, thus representing a significant limitation in achieving the real genetic profile of this pathology. It is difficult to highlight with certainty the genetic mechanism that could have generated the appearance of a nonsyndromic heart defect, due to gene heterogeneity. The dominant multigenic model must be taken into account, which in the context of an individual susceptibility may determine a specific pathological condition.

Instituting indications, as well as genetic diagnosis methods in newborns with a congenital heart defect, will lead to the development of a genetic protocol specific for the neonatal intensive care units. The continuous development of genetic diagnosis techniques has materialized through the identification of new genes with direct involvement in the occurrence of congenital heart defects. Thus, through the information generated by genetic testing, genetic counseling and recurrence risk determination become increasingly important and relevant. This proves once again the role of a multidisciplinary approach in medicine, both through diagnosis and therapy.