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POSITIVITY OF GENOMIC TESTS IN PATIENTS WITH CONGENITAL HEART DEFECTS IN A TERTIARY HOSPITAL

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Abstract: Introduction: Congenital heart diseases consist of macroscopic structural anomalies of the heart or great vessels that generate functional repercussions on the individual. They currently represent the most common birth defect, with an incidence of approximately 1%-1.2%[1], accounting for approximately one third of all major congenital anomalies. Genetic investigation of patients diagnosed with congenital heart disease has great potential for better understanding the disease, establishing a prognosis and personalized medical treatment, in addition to the possibility of genetic counseling and screening for family members.
Objectives: Based on this panorama, the study seeks to determine the positivity of genetic tests performed on patients with congenital heart defects at InCor-HCFMUSP, a tertiary hospital.
Methodology: This is a single-center prospective study, one of the aspects of a larger project that aims to incorporate genomics into cardiovascular diseases through genomic sequencing and clinical analysis.
laboratory of the subjects. We will analyze the results of 200 patients with congenital heart defects followed at the outpatient clinic and congenital heart disease ward at InCor-HCFMUSP to perform genomic sequencing.
Keywords: Congenital heart disease, genetics, genome

INTRODUCTION

Congenital heart diseases consist of macroscopic structural anomalies of the heart or great vessels that generate functional repercussions on the individual. They currently represent the most common birth defect, with an incidence of approximately 1%-1.2% [1], accounting for approximately one third of all major congenital anomalies. Despite advances in the clinical and surgical treatment of these patients, in Brazil, cardiac malformations still represent the second cause of infant mortality[3]. Therefore, it is essential to delve deeper into the etiological causes of these malformations.

Congenital heart diseases are characterized by enormous complexity and diversity that can be attributed to multifactorial etiologies, involving environmental and genetic exposures. Epidemiological studies suggest that genetic or environmental causes can be identified in approximately 18% to 36%[17] of cases. From a genetic point of view, some already established etiologies include aneuploidies, copy number variations (CNVs), variants in single genes, complex chromosomal abnormalities and submicroscopic rearrangements[6]. Several genes are related to the development of the heart and the embryo as a whole, so congenital heart disease can be an isolated malformation or be associated with a syndromic condition.

Syndromic congenital heart diseases, that is, those characterized by the presence of extra-cardiac manifestations, represent approximately 30% of cases of congenital cardiac malformations[4]. Such cases are commonly related to cases of aneuploidies, translocations, deletions and mutations in single genes, however the genetic mechanisms are still not completely understood in 80% of cases of congenital heart disease[9]. Numerical changes constitute the first genetic cause to be associated with syndromic conditions of congenital heart disease, of which trisomies of chromosomes 13 (Patau Syndrome), 18 (Edwards Syndrome) and 21 (Down Syndrome) stand out, in addition to monosomy X (Turner syndrome). In relation to CNVs, 22q11.2 deletion syndrome (DiGeorge syndrome) stands out, considered the second most common chromosomal alteration related to cardiac malformations[10]. Other deletions worth highlighting are the 1p36 deletion[11], 7q11.2 deletion (Williams-

Beuren Syndrome)[12], terminal deletion of 11q (Jacobsen Syndrome)[13], deletion or duplication of 1q21.1[14] and deletion of 8p23.1[15].

In Brazil, despite the incidence of congenital heart disease following the global incidence, there is still a lack of solid data on genetic relationships with the pathogenesis of heart disease. In this sense, the study of molecular diagnosis based on genetic sequencing will contribute to a better understanding of these pathologies, as well as assisting in the diagnostic process. Carrying out an accurate diagnosis, in turn, will contribute to detailing the prognosis, understanding the mechanisms of the disease and genetic counseling for the family, in addition to the management of extracardiac manifestations in syndromic cases.

STUDY OBJECTIVES

PRIMARY OBJECTIVE:

The general objective of the study is to determine the positivity of genomic tests in patients with congenital heart disease.

SECONDARY OBJECTIVES

1. Characterize the clinical and epidemiological profile of patients with congenital heart disease.
2. Characterize pathogenic variants associated with cardiac malformations in terms of their spectrum and frequency.
3. Offer genetic counseling to family members.

METHODOLOGY

TYPE OF STUDY

This is a single-center, observational and prospective study, being one of the aspects of a larger project ("Precision health: correlation between genomic, epidemiological, clinical and family profiles in cardiovascular diseases").

ETHICAL ASPECTS

All volunteers agreed to participate in the project, signing the free and informed consent form (TCLE). Patients under 18 years of age agreed to participate in the study by signing the Free and Informed Assent Form (TALE) written in accessible language according to their age group. The parents and guardians of the minor will sign the Free and Informed Consent Form (ICF).

Participants did not receive any financial supplement for participation.

INCLUSION AND EXCLUSION CRITERIA

228 patients were selected from the outpatient clinic and congenital heart disease ward at InCor-HCFMUSP with a clinical diagnosis of congenital heart disease who met one of the following criteria:

- syndromic phenotypic characteristics
- isolated congenital heart disease with positive family history
- complex heart diseases
- conotruncal anomalies
- laterality defects (e.g. situs inversus, dextrocardia, isomerism)

No age limit was determined.

Patients with previous genetic studies will not be included in the study.

Patients with suspected aneuploidy will not be included.

PROCEDURES

Patient recruitment will be carried out by doctors from outpatient clinics or wards who will identify the patients' eligibility criteria and forward them to the project team. A team composed of a cardiologist and research assistant will check the clinical eligibility criteria, obtain the informed consent form, complete the clinical form, collect the peripheral blood sample and provide pre-test genetic counseling.

The sample will be sent to the laboratory for DNA extraction and complete genome sequencing. The raw sequencing data will be sent within 40 business days to the laboratory's bioinformatics team to perform data quality control, raw data analysis and data storage in a genomic bank.

Then, a team composed of a molecular biologist, cardiologist and geneticist will analyze the data and prepare the report. The report will be delivered to the patient by the project team, who will also offer post-test genetic counseling. It is important to emphasize that the proponent will participate in all stages of the process from pre-test investigation, analysis of genetic results to selection of candidate variants and preparation of medical and post-test reports, which will include discussions with the multidisciplinary team of the Medicine Service of InCor Precision Cardiology, with health professionals involved in patient care, with patients and family members, when applicable.

GENETIC ANALYSIS

Genome sequencing will be performed on all patients (WGS - *Whole Genome Sequencing*). In cases of suspected diagnosis in syndromic conditions, the MLPA test may be performed to identify insertions and deletions in the chromosomal region 22q11 as initial *screening*; if negative, the complete genome will be performed.

All participants will receive a genetic report, regardless of the test result, with pre- and post-test genetic counseling

GENETIC REPORTS

The results of genomic sequencing will be communicated based on genetic reports prepared in accordance with the Technical Opinion of the Brazilian Society of Medical Genetics and Genomics on Genetic Testing.

The classification of genetic variants will be done using the criteria established by the ACMG (*American College of Medical Genetics*)[16]. Variants will be reported as Pathogenic, Possibly Pathogenic and Variant of Uncertain Significance. Possibly benign or benign variants will not be reported. Secondary findings will only be reported if they are of interest to the patient. The doctor will be responsible for informing the participant during care about the possibility of identifying pathogenic genetic variants not associated with their primary disease and the patient must choose whether they wish to be notified about incidental findings.

FINAL RESULTS

LITERATURE REVIEW AND UPDATE

To carry out the study, a review of the initial literature was carried out. Articles were searched in PubMed using the English terms for Congenital Heart Disease, Genetics and Genome, with the aim of generating greater proximity to the subject and gathering results from previous studies in relation to the positivity of genomic tests in congenital heart diseases.

Based on the analysis of previous studies, a positivity rate ranging from 20%[6] to 40%[5] was found, but with few specific studies in tertiary hospitals.

INCLUSION OF PATIENTS

The identification of potential study patients who met the inclusion criteria was carried out through screening by doctors from the InCor outpatient clinic and Congenital Heart Diseases ward. To date, 228 patients have been identified and recruited for the study.

Of the main etiologies, Tetralogy of Fallot stands out with 17.7% (n = 40), Ebstein's Anomaly with 8.4% (n = 19), Coarctation of the aorta with 6.6% (n = 15) and Left Heart Hypoplasia Syndrome with 9.7% (n = 22). Furthermore, defects such as atrial septal defect and interventricular septal defect were present in 115 and 74 patients, respectively.

Furthermore, of the 228 patients with congenital heart disease, 34 patients with cardiomyopathy were included, of which 18 classified as dilated cardiomyopathy, 13 as hypertrophic cardiomyopathy and 3 as non-compacted myocardium.

PRE-TEST GENETIC COUNSELING

After the identified patients were referred by the outpatient and infirmary teams, the participants were directed to the laboratory and underwent pre-test genetic counseling with the project team. At this time, all participants underwent a study eligibility check, obtained an Informed Consent Form or Assent, pre-test genetic counseling and blood sample collection.

CARRYING OUT GENOMIC SEQUENCING

All patients diagnosed with congenital heart disease who were included in the study (228) had genomic sequencing performed (*WGS - Whole Genome Sequencing)*. In the case of patients diagnosed with cardiomyopathy (34 patients), exome sequencing was performed (*WES - Whole Exome Sequencing*).

DATA ANALYSIS

The analysis of raw genomic sequencing data presented impasses due to limitations of the analysis software of the laboratory's bioinformatics team, due to the magnitude of the genomic sequencing data. Alternatives are being worked on to carry out the analysis of raw data and to continue the study stages, such as generating the VCF (*variant call file)* and preparing reports relating to congenital heart diseases by the project team.

Regarding the analysis of exome sequencing for cases of cardiomyopathies, the raw data was analyzed by the project's bioinformatics team, with subsequent generation of the VCF annotated by a team of trained geneticists and data storage in the REDCAP system.

PREPARATION OF REPORTS

The reports were prepared by a molecular biologist, cardiologist and geneticist.

The classification of genetic variants was carried out using the criteria established by the ACMG (American College of Medical Genetics) [16]. The variants were reported as Pathogenic, Possibly Pathogenic and Variant of Uncertain Significance. Thus, positive tests were those that presented variants classified as Pathogenic, Possibly Pathogenic or Variant of Uncertain Significance.

DELIVERY OF REPORTS AND GENETIC COUNSELING

DATA ANALYSIS

To date, 47 genomes of the 228 patients included in the study have been sequenced. Of these, in 26 patients some variant of Pathogenic, Probably Pathogenic or Variant of Uncertain Significance was identified. As a result, negativity was 41%, while the presence of variants was identified in 59% of tests, with 19.2% (n = 5) pathogenic variants, 19.2% (n = 5) Possibly Pathogenic variants and 61.5% (n = 16) Variants of Uncertain Meaning. Therefore,

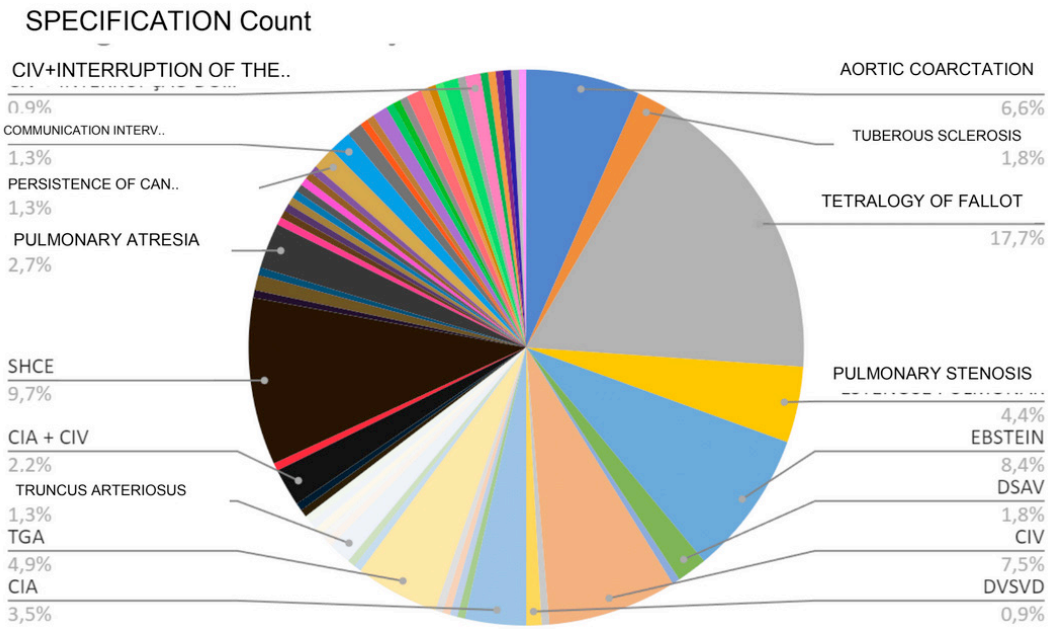

Figure 1: Chart of analyzed heart diseases

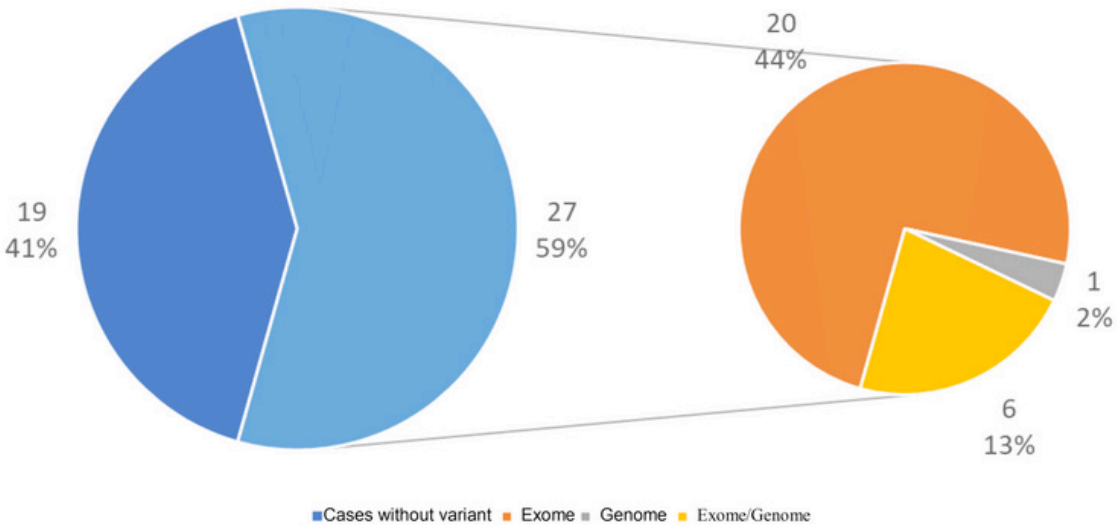

Figure 2: Graph of variants found in tests

a positivity of 21.3% was identified.

Of the tests classified as positive, 44% (n=20) of them had variants that could have been identified in exome sequencing, 13% (n=6) could or could not be identified in the exome depending on the quality of the test and 2% (n=1) would be identified only by the genome.

DISCUSSION

The descriptive analysis of the genomic sequencing results demonstrated a rate of 59% in relation to the presence of variants of uncertain significance, pathogenic or possibly pathogenic. Regarding positivity, that is, the presence of a pathogenic or probably pathogenic variant, the rate was 21.3%. Although the study was carried out in a tertiary hospital, where highly complex

and more serious patients are treated, the positivity rate is in line with previous studies that demonstrated positivity between 18% and 36% [17] depending on the sequencing method.

The study demonstrated that around 15% of patients presented variants in regions analyzed only by the genome or in regions where identification by exome would depend on the analysis and test technique, due to variations such as distance from the edge of the exon with coverage and analysis of CNVs. Therefore, it is important to consider the benefit of genomic versus exomic sequencing when choosing genetic testing in patients with congenital heart disease.

CONCLUSIONS

Thus, it is expected that the results can impact the planning of strategies, increase the diagnostic and prognostic efficacy of patients with congenital heart diseases, since the development of precision medicine provides earlier diagnoses and, consequently, a greater number of therapeutic alternatives.

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