Chromosomal Abnormalities in Patients with Congenital Heart Disease

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Abstract

Background: Chromosomal abnormalities (CAs) are an important cause of congenital heart disease (CHD).

Objective: Determine the frequency, types and clinical characteristics of CAs identified in a sample of prospective and consecutive patients with CHD.

Method: Our sample consisted of patients with CHD evaluated during their first hospitalization in a cardiac intensive care unit of a pediatric referral hospital in Southern Brazil. All patients underwent clinical and cytogenetic assessment through high-resolution karyotype. CHDs were classified according to Botto et al. Chi-square, Fisher exact test and odds ratio were used in the statistical analysis (p < 0.05).

Results: Our sample consisted of 298 patients, 53.4% males, with age ranging from 1 day to 14 years. CAs were observed in 50 patients (16.8%), and 49 of them were syndromic. As for the CAs, 44 (88%) were numeric (40 patients with +21, 2 with +18, 1 with triple X and one with 45,X) and 6 (12%) structural [2 patients with der(14,21), +21, 1 with i(21q), 1 with dup(17p), 1 with del(6p) and 1 with add(18p)]. The group of CHDs more often associated with CAs was atrioventricular septal defect.

Conclusions: CAs detected through karyotyping are frequent in patients with CHD. Thus, professionals, especially those working in Pediatric Cardiology Services, must be aware of the implications that performing the karyotype can bring to the diagnosis, treatment and prognosis and for genetic counseling of patients and families. (Arq Bras Cardiol. 2013; 101(6):495-501)

Keywords: Heart Defects, Congenital; Chromosome Aberrations; Down Syndrome; Karyotype; Metaphase.

Introduction

The incidence of congenital heart defects ranges from 4-50 per 1,000 births1,2. They are defined as a group of alterations that affect the heart and great vessels2. Depending on the type and severity of the alteration, patients may require different interventions3, and the need for intensive care unit (ICU) admission is frequently observed4. Furthermore, studies have shown the great impact that congenital heart defects have on mortality in children5.

The etiology of cardiac malformations is still little understood6 and their determination is a very important factor for adequate patient management and treatment. Among the known causes of congenital heart disease, chromosomal abnormalities are highlighted7. It was from the second half of the twentieth century, with the development of new cell culture techniques associated with the use of colchicine and hypotonic solutions in the treatment of metaphases, that cytogenetics, i.e., the study of chromosomes, disseminated.

In 1970, Caspersson et al8 developed a staining technique that yielded a more precise identification of each chromosome through its unique more or less intense region staining patterns (bands). Moreover, with the emergence of high-resolution chromosome analysis by Yunis9, in 1981, chromosomes could be investigated at an early stage of mitosis (prometaphase), allowing chromosomal bands to be more detailed10. Several studies have been developed in recent decades, aiming to evaluate the frequency and types of chromosomal abnormalities identified through karyotype in patients with congenital heart disease. The observed rates usually range from 3-18%. However, these studies are mostly retrospective and based on databases3,6,11-22. Moreover, it is noteworthy the virtual lack of studies carried out in Latin America20.

Thus, our study aimed to determine the frequency, type and clinical characteristics of chromosomal abnormalities identified by high-resolution karyotype in a prospective and consecutive sample of patients with congenital heart disease.
Methods

Patients

Our sample consisted of patients from to the studies by Rosa et al\textsuperscript{23} and Zen et al\textsuperscript{24}. They comprised a prospective and consecutive cohort of patients with congenital heart disease hospitalized in the ICU of a pediatric referral hospital in southern Brazil. Only those patients at their first hospitalization were included. The total evaluation period was 1.5 year. This study was approved by the Research Ethics Committee of the hospital and the university. We only included patients whose families agreed to participate in the study.

Clinical protocol

An evaluation form was completed by clinical geneticists for each subject participating in the study. This was accomplished through direct interviews with family members, review of hospital records and clinical evaluation of patients. In our study, we used general data such as gender, patient age, reason for admission, origin and syndromic appearance. As for origin, the patients were divided into those who came from Porto Alegre (the city where the study was carried out), Porto Alegre suburbs, from other cities in the state of Rio Grande South and other states.

The syndromic diagnosis was made before the results of cytogenetic analysis and was defined solely on physical examination, taking into account both quantitative (number of minor and major anomalies) and qualitative (types and pattern of dysmorphic features, presence of neurological alterations) data\textsuperscript{25}. The cardiac diagnosis was obtained from the results of echocardiographic examinations, surgery and/or cardiac catheterization. Congenital heart defects were then defined and classified according to Botto et al\textsuperscript{26}. Furthermore, congenital heart defects were classified as complex and cyanotic.

Cytogenetic study through high-resolution karyotype

A blood sample was collected from each patient, and high-resolution karyotype (≥ 550 bands) was performed according to the modified technique of Yunis\textsuperscript{8}. This technique, unlike conventional karyotyping, allows the chromosomes to be analyzed at a very early stage of mitosis, in prometaphase, when chromosomes are less condensed. Thus, it allows better identification of minor structural chromosomal abnormalities, such as small deletions. In summary, this technique is based on the procedure of cell culture of lymphocytes stimulated with phytohemagglutinin for 72 hours, synchronization with methotrexate/thymidine and GTG-banding staining. The analysis of the slides in each case was performed in an Axioplan Zeiss microscope using a count of 25 metaphase plates, which excludes a degree of mosaicism of up to 12% for a 95% confidence level\textsuperscript{25}.

Statistical Analysis

Data processing and analysis were performed using SPSS for Windows (release 18.0), Microsoft \textregistered Excel 2002 and PEPPI (release 4.0). The statistical analysis used the chi-square and Fisher’s exact two-tailed tests for comparison of frequencies, and odds ratios to assess the association between cardiac defects and chromosomal abnormalities. Values were considered significant when p < 0.05.

Results

General sample data

During a period of one year and six months, 333 patients with congenital heart disease met the criteria for inclusion in the study. However, 31 of them did not participate in the study, due to death (n = 12) or because they had been discharged prior to the application of informed consent (n = 4), or because their parents did not agree to participate (n = 15). Of 302 patients with consent, the karyotype was successfully performed in 298, and these comprised our final sample. Of these 298 patients, 159 (53.4%) were males, ranging in age from one day to 14 years of age, with just over half of them (58.7%) in the first year of life. The main reason for admission was cardiac surgery (76.2%); among the remaining patients, about half was there for cardiac evaluation and half for cardiac catheterization.

As for the origin, 13.8% were from the city of Porto Alegre, 21.1% were from the city suburbs, 55% were from other cities in the state of Rio Grande do Sul and 10.1% were from other states. As for the physical examination, 29.5% of patients were classified as syndromic. Of these, 70.5% had a classic syndrome phenotype.

The anatomical types of cardiac malformation observed are shown in Table 1. Ventricular (VSD) and atrial (ASD) septal defect were the most frequent alterations, each observed in 14.8% of cases. According to the classification of Botto et al\textsuperscript{26}, the main group of observed cardiac alterations was septal defects (29.5% - Table 1). Complex heart disease was observed in 34.6% of cases, and cyanotic disease, in 35.2%.

Chromosomal alterations

Chromosomal abnormalities were observed in 50 subjects (16.8%) and the full trisomy of chromosome 21 was the most frequent of them (n = 40). Of the remaining cases, four had numerical and six structural abnormalities (Figure 1 and Table 1). Although most patients with chromosomal abnormalities were from the countryside of the state of Rio Grande do Sul, there was no statistically significant difference (p = 0.998) when evaluating the presence or absence of these alterations regarding patient origin.

Most patients with chromosomal abnormalities were admitted at the ICU for cardiac surgery (76.2%) and cardiac evaluation (11.7%). The main clinical characteristics of the patients with chromosomal abnormalities are shown in Tables 1 and 2. According to the classification by Botto et al\textsuperscript{26}, the group of heart defects in our sample more often associated with chromosomal abnormalities was atrioventricular septal defect (66.7%, OR: 16.929, 95% CI: 7.434 to 38.55, p < 0.001). Septal malformations were frequent (19.3%); however, they did not show a statistically significant association (OR: 1.284, 95% CI: 0.673 to 2.452, p = 0.448). Right and left obstructive cardiac defects, on the other hand, showed an inverse association, i.e., they were statistically not associated with chromosomal abnormalities.
When evaluating anatomic types belonging to the groups classified according to Botto et al\textsuperscript{26} separately, it was observed that the atrioventricular septal defects (66.7%, OR = 16.929, 95% CI: 7.434 to 38.55, p < 0.001) and ventricular septal defects (31.8%; OR: 2.826, 95% CI: 1.368 to 5.839, p = 0.005) were more often associated with chromosomal abnormalities (Figure 2). All cases of chromosomal alteration associated with atrioventricular septal defect consisted of patients with Down syndrome. On the other hand, D-transposition of the great arteries was statistically not associated with chromosomal abnormalities (none of the cases with this defect had the latter, p = 0.0310 - Table 1). On physical examination, 49 of 50 patients with chromosomal abnormalities were considered syndromic (only the patient with triple X was nonsyndromic - Table 2).

### Discussion

In our review of the literature using the PubMed and SciELO databases, we identified 14 studies similar to ours, which evaluated the frequency and types of chromosomal abnormalities identified through karyotyping in patients with congenital heart disease\textsuperscript{3,6,11-22}. Studies such as the one by Schellberg et al\textsuperscript{26}, which excluded patients with frequent chromosomal alterations (such as trisomy 21) from the sample, were not included in our analysis. The vast majority of similar studies were developed in the United States and Europe. Only one (by Amorim et al\textsuperscript{20}) was carried out in Latin America and Brazil. However, it is noteworthy that, unlike us, most studies (including the one by Amorim et al\textsuperscript{20}) were performed retrospectively, based mainly on databases. Due to this fact, the karyotype was not performed in a standardized way in many studies (the testing often appeared to be limited primarily to the cases with suspected chromosomal abnormality)\textsuperscript{12,18}. Furthermore, our study was the only one in which a geneticist assessed and carried out the syndromic classification of patients based on data from the physical and dysmorphologic assessment (Table 2).

The frequency of chromosomal abnormalities identified through karyotyping in our study (16.8%) was similar to that found in the studies of Ferencz et al\textsuperscript{11}, Pradat\textsuperscript{13}, Harris et al\textsuperscript{19} and Amorim et al\textsuperscript{20}, who found rates of 12.9-23.1%. Significant differences were observed in relation to the work of Stoll et al\textsuperscript{12}, Hanna et al\textsuperscript{14}, Goodship et al\textsuperscript{15}, Grech and Gatt\textsuperscript{3}, Meeberg et al\textsuperscript{16}, Roodpeyma et al\textsuperscript{6}, Bosi et al\textsuperscript{17}, Calzolari et al\textsuperscript{18} Dadvand et al\textsuperscript{21} and Hartman et al\textsuperscript{22}, who found rates of 3-12.1% (p < 0.05). These were characterized by having distinct samples, both regarding the number and the clinical characteristics of their patients. In our sample, the frequency of chromosomal abnormalities did not differ regarding the origin of patients, suggesting that perhaps there was no selection

| Table 1 - Congenital heart defects, classified by Botto et al\textsuperscript{26} and karyotype findings observed in patients from the sample |
|---------------------------------------------------------------|
| **Heart defects** | **Normal** | **Chromosomal alteration** | **Total** |
| | karyotype | **Numerical** | **Structural** | |
| | | +21 | +18 | XXX | 45,X | dup(17p) | add(18p) | i(21q) | del(6p) | der(14;21),+21 |
| Outflow tract defects | 56 | 6 | 1 | 1 | 64 | 21.5 |
| Tetralogy of Fallot | (26) | (6) | (1) | (1) | 34 |
| Atrioventricular septal defect | 11 | 21 | 1 | 33 | 11.1 |
| Ebstein’s Anomaly | 3 | 1 | | | 4 | 1.3 |
| Left obstructive defects | 45 | 1 | | 47 | 15.8 |
| Aortic coarctation | (28) | (1) | | (29) |
| Aortic valve stenosis | (4) | (1) | | (5) |
| Septal defects | 71 | 11 | 1 | 1 | 1 | 88 | 29.5 |
| Ventricular septal defects | (30) | (9) | (1) | (1) | (1) | (1) | (1) | (44) |
| Atrial septal defects | (41) | (2) | (1) | | | | | (44) |
| Other heart defects | 62 | 40 | 2 | 1 | 1 | 1 | 1 | 1 | 2 | 298 | 100 |

* +18: full trisomy of chromosome 18; +21: full trisomy of chromosome 21; 45,X: monosomy X; add (18p): additional material by the end of the short arm of chromosome 18; del (6p): deletion of the short arm of chromosome 6; der(14;21),+21: trisomy of chromosome 21 secondary to translocation between chromosomes 14 and 21; dup (17p): duplication of the short arm of chromosome 17; i(21q) Down syndrome secondary to isochromosome of the long arm of chromosome 21; XXX: trisomy X.*
Table 2 - Classification according to the syndrome characteristics, based only on physical examination

| Chromosomal alteration | Classic syndrome | Undefined Syndrome | Heart disease + dysmorphism | Isolated heart disease | Total |
|------------------------|------------------|--------------------|---------------------------|-----------------------|-------|
| +21                    | 40               | 0                  | 0                         | 0                     | 40    |
| +18                    | 1                | 1                  | 0                         | 0                     | 2     |
| XXX                    | 0                | 0                  | 1                         | 0                     | 1     |
| 45,X                   | 1                | 0                  | 0                         | 0                     | 1     |
| der(14;21)+21          | 2                | 0                  | 0                         | 0                     | 2     |
| i(21q)                 | 1                | 0                  | 0                         | 0                     | 1     |
| dup(17p)               | 0                | 1                  | 0                         | 0                     | 1     |
| del(6p)                | 0                | 1                  | 0                         | 0                     | 1     |
| add(18p)               | 0                | 1                  | 0                         | 0                     | 1     |
| Total                  | 44               | 5                  | 1                         | 0                     | 50    |

+18: full trisomy of chromosome 18; +21: full trisomy of chromosome 21; add (18p): additional material by the end of the short arm of chromosome 18; 45,X: monosomy X; del (6p): deletion of the short arm of chromosome 6; der(14;21);+21: trisomy of chromosome 21 secondary to translocation between chromosomes 14 and 21; dup (17p): duplication of the short arm of chromosome 17; i(21q) Down syndrome secondary to isochromosome of the long arm of chromosome 21; XXX: trisomy X.
associated with chromosomal abnormalities. That is, there was no difference between the frequency of patients with severe alterations that came from the city capital compared to those that came from the countryside of the state.

Down syndrome, especially in individuals with full trisomy of chromosome 21, was the most frequently observed chromosomal abnormality in our series of patients (14.4%), which is consistent with the literature. The full trisomy of chromosome 18 was the second most frequent one and recurrent among patients with congenital heart disease. Another condition often described in the studies, but absent from our sample was trisomy of chromosome 13.

Regarding structural changes, only the deletion of the short arm of chromosome 6 was also observed in another study. The patient with duplication of the short arm of chromosome 17 has been described in detail by Paskulin et al. The frequency of structural alterations observed in our sample (12%) was similar to the one in most other studies (4.2 to 16.7%), differing only in relation to Ferencz et al, who found a lower frequency (4.4%). It is noteworthy that this study was the oldest, developed in the early 1980s, at a time when the high-resolution technique was being described. In our series, in spite of the count of 25 metaphase slides (which excludes a degree of mosaicism of up to 12% for a 95% confidence level), no cases of mosaicism were observed. These have been reported in low frequencies only in the studies by Goodship et al and Hartman et al. Nevertheless, the overall frequency of chromosomal abnormalities was lower than the one in our study.

As for the association of chromosomal alterations with types of heart defects, we observed, according to Botto et al, a very significant association with atrioventricular septal defect, at the expense mainly of patients with Down syndrome (our frequency was 66.7%). This finding is consistent with the literature, which describes rates of 40-50%. Thus, when assessing a patient with atrioventricular septal defect, there is a high probability (of up to one in two) that the patient has Down syndrome.

It was noteworthy the lack of association of chromosomal alterations with some defect groups, such as, left and right obstructive defects (including defects such as pulmonary stenosis and aortic coarctation). Alone, that was observed only in relation to an outflow tract defect, D-transposition of the great arteries. Although we did not observe an association between chromosomal abnormalities and septal defects, separately we found an association with ventricular septal defects. In the literature, chromosomal abnormalities have been described in approximately 4.6 to 18.2% of patients with ventricular septal defects and in our sample, this frequency was 31.8%.

Although our study was the only one that had the syndromic classification description made by a clinical geneticist, based only on the dysmorphologic physical examination findings of patients, our frequency of syndromic patients (29.5%) was similar to that reported by Calzolari et al and Amorim et al. Unlike ours, in these studies the patients were retrospectively divided into syndromic or not after the karyotype evaluation results and the presence of abnormalities in other organs. Chromosomal abnormalities
are frequent in syndromic individuals. In our sample, only one of 50 patients with chromosomal abnormalities was considered nonsyndromic, who was an individual with triple X syndrome. This finding is consistent with the literature, as individuals with triple X often go unnoticed amid the general population, as they usually do not have associated major findings. In our case, we cannot rule out the possibility that the association with congenital heart disease may be coincidental, as both conditions are relatively frequent (triple X occurs in one in 1,000 live female births)\(^\text{11}\).

In spite of the advances in cytogenetics that occurred in recent decades with the development of molecular techniques, such as fluorescent in situ hybridization (FISH) and comparative genomic hybridization (CGH), karyotyping remains a crucial and basic tool in genetic evaluation\(^\text{10}\).

In our country, it is one of the few tests available for the evaluation of patients treated by the Unified Health System, the public health system of Brazil. Despite its limitations in detecting small chromosomal rearrangements such as microdeletions or microduplications, the frequency of chromosomal abnormalities detected by karyotyping in patients with congenital heart disease is remarkable. Thus, professionals, especially those working in pediatric cardiology services, should be aware of the implications that karyotype assessment can bring, both for the diagnosis, treatment and prognosis of these patients, as well as for genetic counseling of their families.

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### Author contributions
Conception and design of the research, Acquisition of data, Analysis and interpretation of the data, Statistical analysis, Obtaining funding and Writing of the manuscript: Trevisan P, Zen TD, Rosa RFM, da Silva JN, Koshiyama DB, Paskulin GA, Zen PRG; Critical revision of the manuscript for intellectual content: Trevisan P, Rosa RFM, Paskulin GA, Zen PRG

### Potential Conflict of Interest
No potential conflict of interest relevant to this article was reported.

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