Original Article

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 births[1,2]. They are defined as a group of alterations that affect the heart and great vessels[2]. Depending on the type and severity of the alteration, patients may require different interventions[3], and the need for intensive care unit (ICU) admission is frequently observed[4]. Furthermore, studies have shown the great impact that congenital heart defects have on mortality in children[5].

The etiology of cardiac malformations is still little understood[6] 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 highlighted[7]. 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., is the study of chromosomes, disseminated.

In 1970, Caspersson et al[8] 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 Yunis[9], in 1981, chromosomes could be investigated at an early stage of mitosis (prometaphase), allowing chromosomal bands to be more detailed[10].

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 databases[3,6,11-22]. Moreover, it is noteworthy the virtual lack of studies carried out in Latin America[20].

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[23] and Zen et al[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[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[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 ($\geq$ 550 bands) was performed according to the modified technique of Yunis[9]. 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 Axioskop Zeiss microscope using a count of 25 metaphase plates, which excludes a degree of mosaicism of up to 12% for a 95% confidence level[27].

Statistical Analysis

Data processing and analysis were performed using SPSS for Windows (release 18.0), Microsoft ® Excel 2002 and PEPI (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[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, Tables 1 and 2). 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 50 patients with chromosomal abnormalities are shown in Tables 1 and 2. According to the classification by Botto et al[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.

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Table 1 - Congenital heart defects, classified by Botto et al[26] and karyotype findings observed in patients from the sample

Heart defects	Normal karyotype	Numerical +21	+18	XXX	45,X	Structural dup(17p)	add(18p)	i(21q)	del(6p)	der(14;21),+21	Total	(%)
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			1						47	15.8
Aortic coarctation	(28)	(1)									(29)	
Aortic valve stenosis	(4)				(1)						(5)	
Septal defects	71	11	1	1		1	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										62	20.8
Total	248	40	2	1	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.

When evaluating anatomic types belonging to the groups classified according to Botto et al[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 karyotype in patients with congenital heart disease[3,6,11-22]. Studies such as the one by Schellberg et al[28], 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[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[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)[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[11], Pradat[13], Harris et al[19] and Amorim et al[20], who found rates of 12.9-23.1%. Significant differences were observed in relation to the work of Stoll et al[12], Hanna et al[14], Goodship et al[15], Grech and Gatt[3], Meberg et al[16], Roodpeyma et al[6], Bosi et al[17], Calzolari et al[18] Dadvand et al[21] and Hartman et al[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
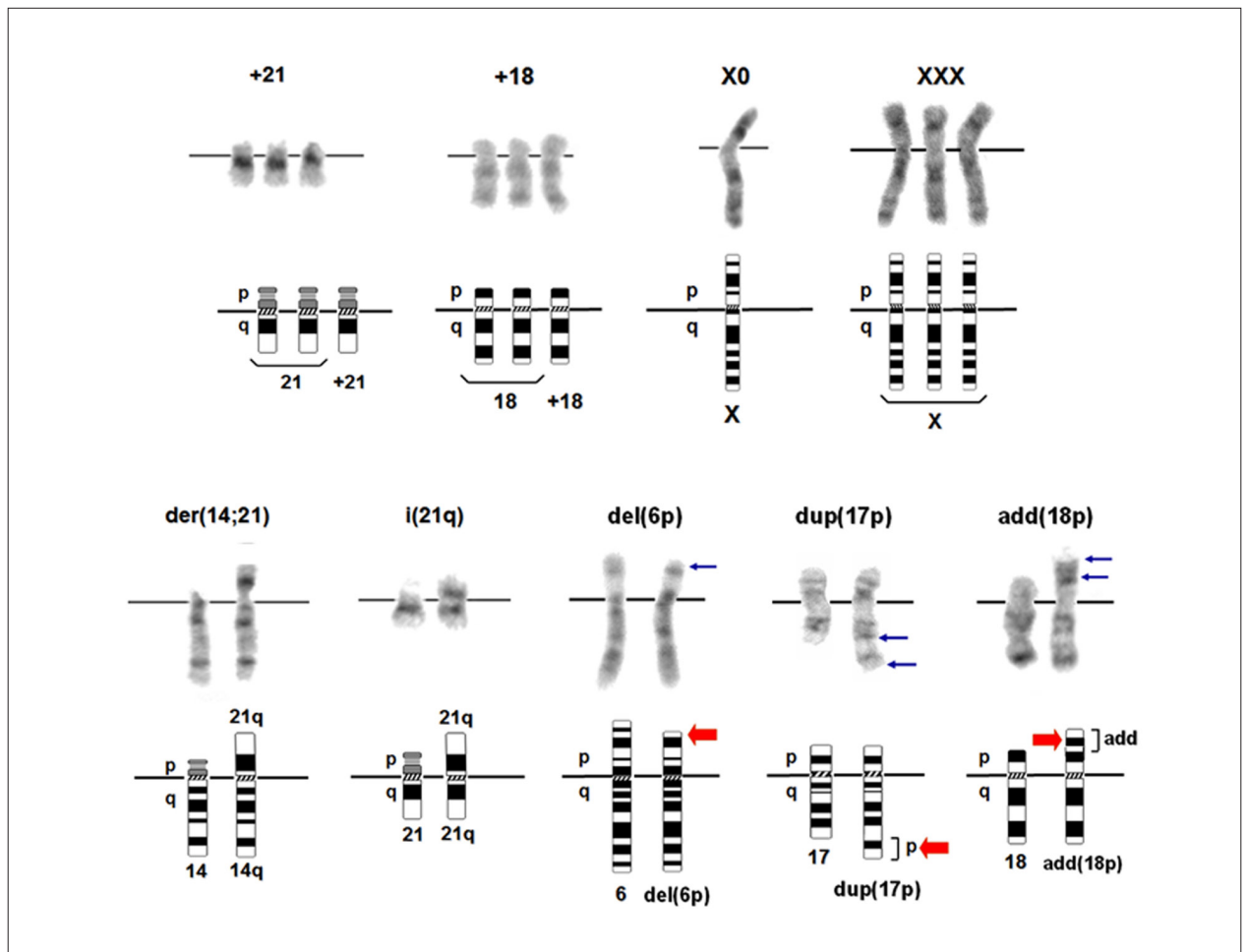
Figure 1 - *Partial GTG-banded karyotype and ideograms of chromosomal abnormalities observed in the sample.*

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.

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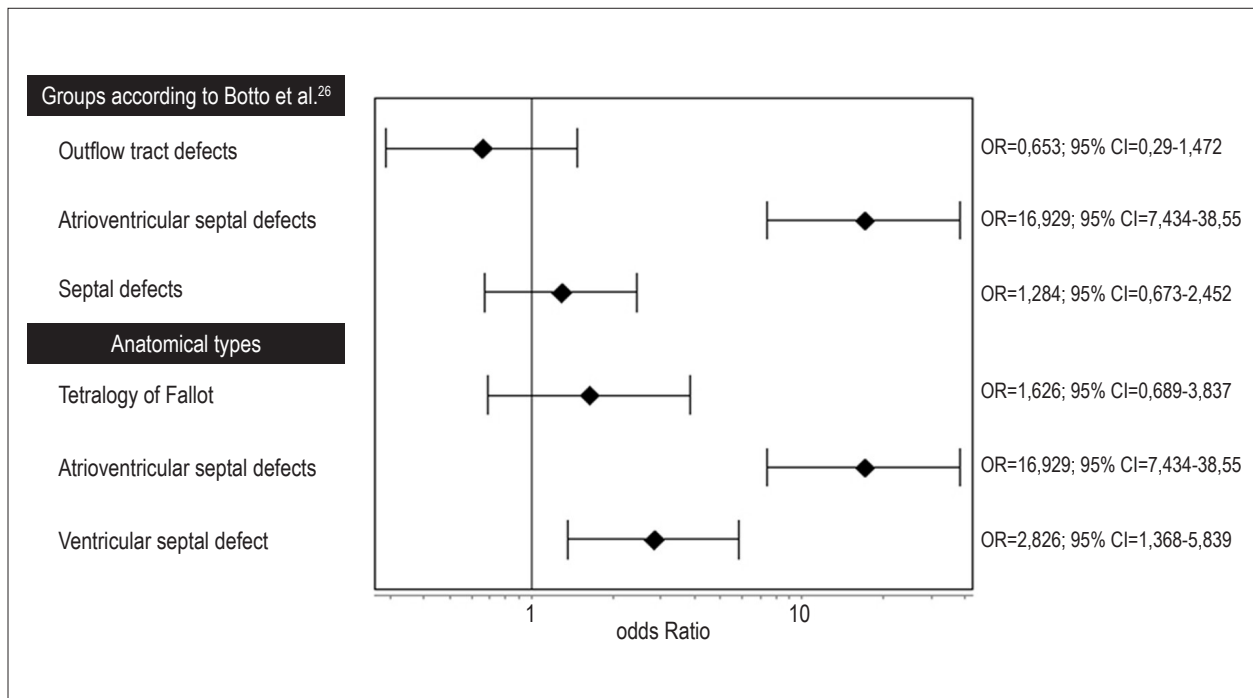
Figure 2 - *Chart showing odds ratios with confidence intervals (95%CI) of the main groups and anatomical types of heart defects observed in the sample regarding the presence of chromosomal abnormalities.*

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[11-13,16-22].

Regarding structural changes, only the deletion of the short arm of chromosome 6 was also observed in another study[22]. The patient with duplication of the short arm of chromosome 17 has been described in detail by Paskulin et al[29]. 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[11], 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[10]. 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)[27], no cases of mosaicism were observed. These have been reported in low frequencies only in the studies by Goodship et al[15] and Hartman et al[22]. 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[26], 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%[30]. 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[11,18,19] 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[18] and Amorim et al[20]. 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 have been coincidental, as both conditions are relatively frequent (triple X occurs in one in 1,000 live female births)[31].

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[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.

References

1. Hoffman JI. Incidence of congenital heart disease: I. Postnatal incidence. Pediatr Cardiol. 1995;16(3):103-13.

2. Hoffman JI, Kaplan S. The incidence of congenital heart disease. J Am Coll Cardiol. 2002;39(12):1890-900.

3. Grech V, Gatt M. Syndromes and malformations associated with congenital heart disease in a population-based study. Int J Cardiol. 1999;68(2):151-6.

4. Kapil D, Bagga A. The profile and outcome of patients admitted to a pediatric intensive care unit. Indian J Pediatr. 1993;60(1):5-10.

5. Boneva RS, Botto LD, Moore CA, Yang Q, Correa A, Erickson JD. Mortality associated with congenital heart defects in the United States: trends and racial disparities, 1979-1997. Circulation. 2001;103(19):2376-81.

6. Roodpeyma S, Kamali Z, Ashar F, Naraghi S. Risk factors in congenital heart disease. Clin Pediatr (Phila). 2002;41(9):653-8.

7. Johnson MC, Hing A, Wood MK, Watson MS. Chromosome abnormalities in congenital heart disease. Am J Med Genet. 1997;70(3):292-8.

8. Caspersson T, Lindsten J, Lomakka G, Wallman H, Zech L. Rapid identification of human chromosomes by tv-techniques. Exp Cell Res. 1970;63(2):477-9.

9. Yunis JJ. New chromosome techniques in the study of human neoplasia. Hum Pathol. 1981;12(6):540-9.

10. Smeets DF. Historical prospective of human cytogenetics: from microscope to microarray. Clin Biochem. 2004;37(6):439-46.

11. Ferencz C, Neil CA, Boughman JA, Rubin JD, Brenner JI, Perry LW. Congenital cardiovascular malformations associated with chromosome abnormalities: an epidemiologic study. J Pediatr. 1989;114(1):79-86.

12. Stoll C, Alembik Y, Roth MP, Dott B, De Geeter B. Risk factors in congenital heart disease. Eur J Epidemiol. 1989;5(3):382-91.

13. Pradat P. Epidemiology of major congenital heart defects in Sweden, 1981-1986. J Epidemiol Community Health. 1992;46(3):211-5.

14. Hanna EJ, Nevin NC, Nelson J. Genetic study of congenital heart defects in Northern Ireland (1974-1978). J Med Genet. 1994;31(11):858-63.

15. Goodship J, Cross I, Liling J, Wren C. A population study of chromosome 22q11 deletions in infancy. Arch Dis Child. 1998;79(4):348-51.

16. Meberg A, Otterstad JE, Frøland G, Lindberg H, Sørland SJ. Outcome of congenital heart defects – a population-based study. Acta Paediatr. 2000;89(11):1344-51.

17. Bosi G, Garani G, Scorrano M, Calzolari E, IMER Working Party. Temporal variability in birth prevalence of congenital heart defects as recorded by a general birth defects registry. J Pediatr. 2003;142(6):690-8. Erratum in J Pediatr. 2003;143(4):531.

18. Calzolari E, Garani G, Cocchi G, Magnani C, Rivieri F, Neville A, et al; IMER Working Group. Congenital heart defects: 15 years of experience of the Emilia-Romagna Registry (Italy). Eur J Epidemiol. 2003;18(8):773-80.

19. Harris JA, Francannet C, Pradat P, Robert E. The Epidemiology of cardiovascular defects, part 2: a study based on data from three large registries of congenital malformations. Pediatr Cardiol. 2003;24(3):222-35.

20. Amorim LF, Pires CA, Lana AM, Campos AS, Aguiar RA, Tibúrcio JD, et al. Presentation of congenital heart disease diagnosed at birth: analysis of 29,770 newborn infants. J Pediatr (Rio J). 2008;84(1):83-90.

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21. Dadvand P, Rankin J, Shirley MD, Rushton S, Pless-Mulloli T. Descriptive epidemiology of congenital heart disease in Northern England. Paediatr Perinat Epidemiol. 2009;23(1):58-65.

22. Hartman RJ, Rasmussen SA, Botto LD, Riehle-Colarusso T, Martin CL, Cragan JD, et al. The contribution of chromosomal abnormalities to congenital heart defects: a population-based study. Pediatr Cardiol. 2011;32(8):1147-57.

23. Rosa RF, Pilla CB, Pereira VL, Flores JA, Golendziner E, Koshiyama DB, et al. 22q11.2 deletion syndrome in patients admitted to a cardiac pediatric intensive care unit in Brazil. Am J Med Genet A. 2008;146A(13):1655-61.

24. Zen TD, Rosa RF, Zen PR, Trevisan P, Silva AP, Ricachinevsky CP, et al. Gestational and family risk factors for carriers of congenital heart defects in southern Brazil. Pediatr Int. 2011;53(4):551-7.

25. Neuhäuser G, Vogl J. Minor craniofacial anomalies in Children: comparative study of a qualitative and quantitative evaluation. Eur J Pediatr. 1980;133(3):243-50.

26. Botto LD, Correa A, Erickson JD. Racial and temporal variations in the prevalence of heart defects. Pediatrics. 2001;107(3):E32.

27. Hook EB. Exclusion of chromosomal mosaicism: tables of 90%, 95%, and 99% confidence limits and comments on use. Am J Hum Genet. 1977;29(1):94-7.

28. Schellberg R, Schwanitz G, Grävinghoff L, Kallenberg R, Trost D, Raff R, et al. New trends in chromosomal investigation in children with cardiovascular malformations. Cardiol Young. 2004;14(6):622-9.

29. Paskulin GA, Zen PR, Rosa RF, Manique RC, Cotter PD. Report of a child with a complete de novo 17p duplication localized to the terminal region of the long arm of chromosome 17. Am J Med Genet A. 2007;143A(12):1366-70.

30. Marino B, Digilio MC. Congenital heart disease and genetic syndromes: specific correlation between cardiac phenotype and genotype. Cardiovasc Pathol. 2000;9(6):303-15.

31. Jones KL. Smith's recognizable patterns of human malformation. 6th ed. Philadelphia, PA: Elsevier Saunders; 2006.