22q11.2 Duplication and Congenital Heart Defects

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Introduction

The 22q11.2 deletion syndrome (del22q11 syndrome) (OMIM #188400/ #192430), also known as Velocardiofacial syndrome and DiGeorge syndrome, is considered a very common genetic disease. With an estimated prevalence of 1:2,000-6,000 live births, this syndrome currently represents one of the main known causes of congenital cardiopathy[1]. It is known that the region q11.2 of chromosome 22 presents a non-usual rearrangement, with regions of low-copy number repeats which, during meiosis, predispose to a pairing error between the chromosomes and, consequently, to an unequal crossing-over, which can lead to a deletion (del22q11) or a duplication of the q11.2 rgion[2-4]. The latter condition was recently identified and has been characterized by an extremely variable phenotypic spectrum, which includes the presence, among many abnormalities, of congenital cardiac defects[4-6]. However, the actual frequency of these malformations is still unknown in individuals with the 22q11.2 duplication, as well as the frequency of this duplication in patients with congenital cardiopathy[3].

This study aims at verifying the incidence of the 22q11.2 duplication in a sample of patients with congenital cardiopathy, admitted at a cardiology Intensive Care Unit (ICU) of a pediatric hospital in Brazil.

Methods

The sample consisted of patients hospitalized due to congenital cardiopathy in a cardiology ICU of Hospital da Crianca Santo Antonio (HCSA/ CHSCPA), state of Rio Grande do Sul, Brazil, during a one-year period. Only individuals being admitted for the first time at this ICU were included in the study. The patients were prospectively and consecutively allocated, corresponding to the patients present in the study carried out by Rosa et al[1], which assessed the incidence of del22q11 through high-resolution GTG-banding karyotype analysis (550 bands) and fluorescent in situ hybridization (FISH) technique. The first assessment was carried out in an Axioskop Zeiss microscope, through the analysis of 25 metaphase plaques for each patient. In cases suspected of mosaicism, 100 plaques were used.

The analysis through FISH technique was carried out on the fixed material used for karyotype analysis. In these experiments, a commercially available DNA probe was used (DiGeorge/VCFS Region Probe (TUPLE 1) (Vysis, Abbott Molecular Inc.), through a standard co-denaturation protocol. In each case, 20 metaphase plaques and 100 interphase nuclei were analyzed in an epifluorescence microscope (Zeiss Axio Imager M1), equipped with Texas Red, FITC, DAPI, double and triple filters. In cases suspected of mosaicism, 30 metaphase plaques and 500 interphase nuclei were used in the analysis. Incomplete metaphases, very close to each other and with a high background signal were excluded from the analysis. The same was done with ruptured interphase nuclei, worn out by the chemical treatment, overlapped or with an important background signal. The present study was approved by the Ethics Commitee of our Institution.

Results

Of the initial sample of 235 patients that had been hospitalized for the first time at the cardiologic ICU, 28 were excluded from the study due to death (n=11) or hospital discharge before the assessment (n=4) or due to the refusal of the parents in participating in the study (n=13). Of the remaining 207 individuals, the karyotype analysis could not be carried out in 3.

Therefore, of the 204 patients in the final sample, 29 (14.2%) presented chromosomal abnormalities. None of these alterations, however, involved a duplication of the 22q11.2 region. Of the total number of patients, the FISH technique could be performed in 198 of them and in 4 of them (2%), the 22q11.2 microdeletion was detected. No cases of 22q11.2 microduplication were observed, neither at the analysis of the metaphase plaques, nor at the interphase nuclei analysis.

Discussion

The FISH technique is a method that integrates the use of classic cytogenetics with molecular genetics, allowing the identification of specific chromosomal regions through the use of DNA probes labeled with fluorochromes. The TUPLE1 probe, used in the present study, recognizes the homonymous gene as well as the microsatellites D22S553, D22S609, and

Key Words

Heart defects, congenital; gene duplication; chromosome disorders; mosaicism.

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Manuscript received January 12, 2009; revised manuscript received May 21, 2009; accepted June 22, 2009.

D22S942 located within the region commonly deleted in the del22q11 syndrome. This region also corresponds to the usually duplicated one because, as it was mentioned before, a chromosome pairing error followed by a recombination of the 22q11.2 region can lead to either the loss or the duplication of parts of this chromosomal segment. In theory, these events should occur with equal frequency. However, reports of the 22q11.2 duplication syndrome have been quite rare in comparison with the del22q11 syndrome[4-6].

Some authors have speculated that, probably, the 22q11.2 duplication is being underdiagnosed, due to its great clinical variability and technical difficulties[2-8]. Most of the duplications are microscopic (microduplications) and thus, they escape detection at the routine chromosomal analysis. Moreover, the study of metaphases using the FISH technique is inadequate to rule out the 22q11.2 duplication and the assessment of the interphase nuclei is necessary to obtain an accurate diagnosis[2,3,7]. A possible explanation for that fact is that the lower level of chromatin condensation in interphase nuclei allows a better discrimination of the two close fluorescent signals, evidenced in cases of duplication, differently from what occurs in metaphases, where the chromosomes are more condensed, giving the impression of the presence of a single signal[5].

Although many cases of duplication have been identified in screening tests for patients with suspected del22q11 syndrome[2,4,7], there seems to be only a partial overlap between the phenotypes of both conditions[3-5]. Cardiac defects, especially of conotruncal type, are frequently seen among patients with del22q11 syndrome. On the other hand, the syndrome is considered one of the most frequent known causes of congenital cardiopathy, with a frequency that ranges from 1-19%[1]. In the present study, this frequency was 2% and there were no cases of 22q11.2 duplication. Cardiac defects have been described in 1/5 of patients with this duplication (regarding this total number, we also considered reports presented as Abstracts, which included a patient with aortic coarctation; one with mitral and aortic insufficiency; one with Tetralogy of Fallot and one with an unknown cardiac defect)[5,6]. Table 1 shows the different types of cardiac malformations reported in patients with the 22q11.2 duplication. The conotruncal-type malformations (that affect the heart outflow tract) are the most prevalent ones, corresponding to around 50% of the cases[2,7].

However, this cardiac defect frequency, as mentioned before, can represent a bias, as many of the patients with the duplication were identified in samples of individuals with suspected del22q11 syndrome. This is in agreement with the low frequencies or even the lack of patients with the duplication among the individuals with clinical findings of del22q11 syndrome[2,6]. Sivertsen et al[9], for instance, did not find any case of 22q11.2 duplication, among 169 patients with cleft palate, a frequent finding in del22q11 syndrome (in this study, the frequency of deletion was 1.2%). Perhaps the best clinical characterization of these patients was made by Ou et al[4] who, after performing the evaluation of 7,000 cases, referred due to several clinical indications, using the comparative genomic hybridization by microarray (aCGH), verified the presence 22q11.2 duplication in 19 patients. These patients were mainly characterized by a mild and highly variable phenotype that included craniofacial (upslanting palpebral fissures, flattened and wide nasal root, micrognathia, posteriorly rotated ears and preauricular pits) and limb dysmorphism (such as fifth finger clinodactyly and single palmar crease), delayed neuropsychomotor and speech development, hearing deficit and behavioral disorders. Cardiac alterations were observed in only one patient (see Table 1)[4].

Table 1 - Type and frequency of the different cardiac defects described in patients carrying the 22q11.2 duplication reported in the literature.

Cardiac defects	Ensenauer and cols. (2003)[2]		Yobb and cols. (2005)[7]		Sparkes and cols. (2005)[3]		De La Rochebrochard and cols. (2006)[5]	Ou and cols. (2008)[4]	Laitenberger and cols. (2008)[8]	Total
	Pt.1	Pt.2	Pt.1	Pt.2	Pt.1	Pt.2				
TOF	+		+		+					3
LHH		+		+		+				3
TGA							+		+	2
AAI		+								1
Single atrium							+			1
DORV							+			1
TAPVN							+			1
PLSVC							+			1
IVC							+			1
PFO								+		1
ARSA								+		1
Ebstein									+	1

TOF - Tetralogy of Fallot; LHH - left heart hypoplasia; TGA - transposition of great arteries; AAI - aortic arch interruption; DORV - Double outlet right ventricle; TAPVR - Total anomalous pulmonary venous return ; PLSVC - Persistent left superior vena cava; IVC - interventricular communication; PFO - patent foramen ovale ; ARSA - anomalous right subclavian artery; Ebstein - Ebstein's anomaly.

Conclusion

Although our findings are limited to a population of individuals with congenital cardiopathy, admitted at a cardiology ICU, they, in addition to others previously published in the literature, indicate that very possibly, the phenotype of individuals with the reported 22q11.2 duplication is not representative of this condition. Cardiac defects do not seem to be a major finding in these individuals, contrarily to what occurs in patients with the del22q11 syndrome.

Acknowledgements

We thank CAPES (Coordination for the Improvement of Higher Education Personnel) for the scholarship received.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

This study was funded by UFCSPA, University of Colorado Denver (USA) and CAPES.

Study Association

This article is part of the thesis of master degree submitted by Rafael Fabiano Machado Rosa, Tatiana Roman e Giorgio Adriano Paskulin, from Programa de Pós-graduação em Patologia da Unversidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA).

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