22q11.2 Deletion in Patients with Conotruncal Heart Defect and del22q Syndrome Phenotype

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Summary

Background: The 22q11.2 deletion syndrome is the most frequent human microdeletion syndrome. The phenotype is highly variable, being characterized by conotruncal heart defect, facial dysmorphisms, velopharyngeal insufficiency, learning difficulties and mental retardation.

Objective: The objective of this study was to investigate the frequency of deletion 22q11.2 in a Brazilian sample of individuals with isolated conotruncal heart defect and 22q11.2 deletion syndrome phenotype.

Methods: Twenty-nine patients were studied by classical cytogenetics, by fluorescence *in situ* hybridization (FISH), and by molecular techniques.

Results: Cytogenetic analysis by G-banding revealed a normal karyotype in all patients except one who presented a 47,XX,+idic(22)(q11.2) karyotype. Using molecular techniques, a deletion was observed in 25% of the patients, all exhibiting a 22q11.2 deletion syndrome phenotype. In none of the cases the deletion was inherited from the parents. The frequency of 22q11.2 deletion was higher in patients with the clinical spectrum of the 22q11.2 deletion syndrome than in patients with isolated conotruncal heart defect.

Conclusion: Investigating the presence of the deletion and its correlation with the patients' clinical data can help the patients and their families to have a better genetic counseling and more adequate clinical follow-up. (Arq Bras Cardiol 2009;92(4):289-293)

Key words: Chromosome deletion; phenotype; heart defects, congenital; genetic markers.

Introduction

For many years, the DiGeorge (DGS), velocardiofacial (VCFS) and conotruncal and facial anomaly (CAFS) syndromes were considered distinct syndromes. However, as they share the same etiology, i.e., deletion of the region q11.2 of chromosome 22, they are currently classified as variations of the same clinical spectrum, with phenotype overlap and variable expressivity, called the 22q11.2 deletion syndrome[1]. One of the most frequent characteristics of this syndrome is the presence of conotruncal heart malformation, but there can also be other alterations, such as cleft palate, hypoplasia of the thymus and parathyroid gland, facial dysmorphisms, nasal voice, learning difficulties, psychiatric diseases and mild mental retardation[2].

The frequency of the 22q11.2 deletion is of approximately 1:4000 livebirths[3], and it is present in about 90% of patients with the phenotype of the syndrome[4]. This deletion is sporadic in approximately 90% of the cases and inherited from one of the parents in 10%[5]. The great majority of patients (87%) present a 3Mb (megabase) deletion[4]. The deletion is not detectable by classical cytogenetics (karyotype); for this purpose, the use of other techniques, such as fluorescence *in situ* hybridization (FISH) and polymorphic DNA markers, is required.

The identification of a sporadic deletion implies in a low recurrence risk (1 to 3%), whereas for an inherited deletion, the risk of transmission is 50%[1,6]. Therefore, cytogenetic and molecular investigations in affected subjects and their parents are essential for an accurate diagnosis, as well as for adequate genetic counseling.

The objective of this study was to evaluate the chromosomal constitution and the frequency of the 22q11.2 deletion in patients with isolated conotruncal heart defect and in carriers of the complete clinical spectrum of the 22q11.2 deletion syndrome. Whenever the deletion was present, we investigated whether it was inherited or not. Additionally, we compared the FISH and the polymorphic DNA marker methodologies as to their efficiency in detecting the 22q11.2 deletion.

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Patients

The sample consisted of 29 patients (Table 1). The clinical inclusion criteria were: (I) conotruncal heart malformation associated with other clinical aspects of the 22q11.2 deletion syndrome (13 patients), (II) characteristic 22q11.2 deletion syndrome phenotype, without a heart defect (10 patients) and (III) isolated conotruncal heart malformation (6 patients). Most of the patients came from the nursery and the Medical Genetics Center of *Hospital São Paulo* and the others were referred from other hospitals in São Paulo. Seventeen families were constituted by the proband and his (her) parents, seven by the proband and his (her) mother, and five by the proband alone.

The patients' parents, whenever available, were studied by the DNA marker technique as well and, if the child had the deletion, they were also studied by FISH, so as to identify whether the deletion was inherited or not. This study was approved by the Research Ethics Committee of *Universidade Federal de São Paulo/Hospital São Paulo*. All proband families gave their informed consent for participation in the research.

Methods

Cytogenetic analysis

Lymphocyte cultures were made for karyotype analysis[7]. Fifteen metaphases were analyzed for each individual. The metaphase chromosomes, with a 400-550 chromosome band resolution, were classified according to the ISCN (International

Table 1 - Patients' data

	Patient	Gender	Age at 1st examination	Type of heart defect	NPMD	Deletion 22q11.2
Phenotype of the smihome syndrome and heart defect	S19	M	10y 8m	Pulmonary stenosis	retardation	-
	S26	M	7y 8m	IVC and aortic failure	normal	-
	S48	M	NB	Interruption of the aortic arch	/	+
	S49	M	11y 5m	Fallot's tetralogy	retardation	+
	S52	F	28y 11m	IAC and mitral prolapse	retardation	-
	S69	M	NB	IAC, perimembranous IVC	/	+
	S70	M	NB	Hypoplasia of right ventricle and atresia of pulmonary valve	/	-
	S71	M	NB	IVC and interruption of the aortic arch	/	-
	S72	F	1m	Atrioventricular septal defect, IVC and IAC	retardation	+
	S83	F	NB	Complete atrioventricular septal defect Rastelli's type A	/	-
	S86	M	9y 2m	Persistence of arterial canal, isthmal hypoplasia of the aorta	retardation	-
	S94	F	7y 5m	IVC and pulmonary hypertension	retardation	+
Phenotype of the síndrome syndrome without heart defect	S1	F	12y 9m	-	retardation	-
	S2	F	6y 11m	-	retardation	-
	S4	F	7y 3m	-	retardation	-
	S5	M	5y 10m	-	retardation	+
	S7	F	3y 9m	-	retardation	-
	S10	F	7y 6m	-	retardation	+
	S22	M	4y 11m	-	retardation	-
	S29	F	10y 6m	-	normal	-
	S38	F	6y 10m	-	retardation	-
	S45	M	2y 8m	-	retardation	-
Isolated heart defect	S32	M	11y 5m	Fallot's tetralogy	normal	-
	S35	M	11y 7m	Bicuspid aortic valve	normal	-
	S40	M	16y 1m	Fallot-like interventricular communication	normal	-
	S43	M	8y 11m	Fallot's tetralogy, sub-aortic IVC and stenosis of the right branch of the pulmonary artery	normal	-
	S59	F	3y 8m	Fallot's tetralogy	normal	-
	S65	M	1m	Fallot's tetralogy	/	-
'Cat Eye Syndrome	S77	F	4m	IVC and B-type interruption of the aortic arch	/	-

F - female; M - male; NB - newborn; +: presence; -: absence; /: not determined; y - years; m - months; IVC - interventricular communication; IAC - interatrial communication.

System for Human Cytogenetic Nomenclature) (2005). Additional C-banding and NOR (nucleolus organizer region) staining techniques were used whenever necessary.

Fluorescence *in situ* hybridization (FISH)

The FISH technique was applied to lymphocyte cultures using a commercial probe that results in the simultaneous labeling of the DGS chromosome region that includes *TUPLE1* gene (red labeling) and the terminal region of chromosome 22 as control (green labeling) (Cytocell® - Cambridge) (Figure 1). Twenty metaphases and/or 100 interphase nuclei from each patient were analyzed. In the case with a marker chromosome, a probe for the centromeres of chromosomes 14/22 (*D14Z1/D22Z1* - Vysis®) and cosmid probes (c106e4 e c103a2) for the cat-eye-syndrome critical region were used.

Study by polymorphic DNA markers

DNA was extracted from peripheral blood lymphocytes[8] and the PCR technique[9] was performed using primers for three polymorphic loci located in the usually deleted region: D22S941, D22S944N and D22S264[10-12] (Figure 1). The PCR products were tested on 1% agarose gel to investigate the presence of amplification, and then the size polymorphisms were evaluated on high-resolution denaturing polyacrylamide gel (GeneGel HyRes-Amersham Biosciences®) stained with silver nitrate.

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Results

Of the 29 patients studied, 13 were female and 16 male, with ages ranging from one day to 28 years (Table 1).

Karyotype analysis was performed in 28 of the 29 patients (it was not possible to collect peripheral blood from one of the patients) and all but one presented normal results. One patient (S77) had a 47,XX,+mar karyotype. FISH and C- and NOR-banding techniques were performed and showed that the karyotype was 47,XX,+idic(22)(pter→q11.2::q11.2→pter).

Among the patients with normal karyotype, a 22q11.2 deletion was observed in 25% (7/28) of the patients studied. However, when analyzing the groups separately, the deletion was present in about 42% (5/12) of the patients with the syndrome phenotype associated with a heart defect, in 20% (2/10) of the individuals with the syndrome phenotype, but without a heart defect, and in none (0/6) of the patients with isolated conotruncal heart defect. We observed that none of the deletions was inherited.

Considering the data of the three polymorphic DNA markers together, an informative result regarding the presence of the deletion was obtained in 94% (16/17) of the families consisting of a patient and both parents, in 71% (5/7) of the families consisting of a patient and his (her) mother, and in 25% (1/4) of the cases in which only the proband was studied. Considering all cases, the marker results were informative in 79% of the cases (22/28).

Discussion

The 22q11.2 deletion was not detected by karyotype analysis, reinforcing the idea that this technique is not very efficient to investigate this deletion. However, karyotype examination is necessary to investigate other chromosomal aberrations related to heart defect, as in the case of the patient with the 47,XX,+idic(22)(pter→q11.2::q11.2→pter) karyotype. The initial diagnostic hypothesis for this patient was 22q11.2 deletion syndrome, due to the presence of phenotypic anomalies and type B interruption of the aortic

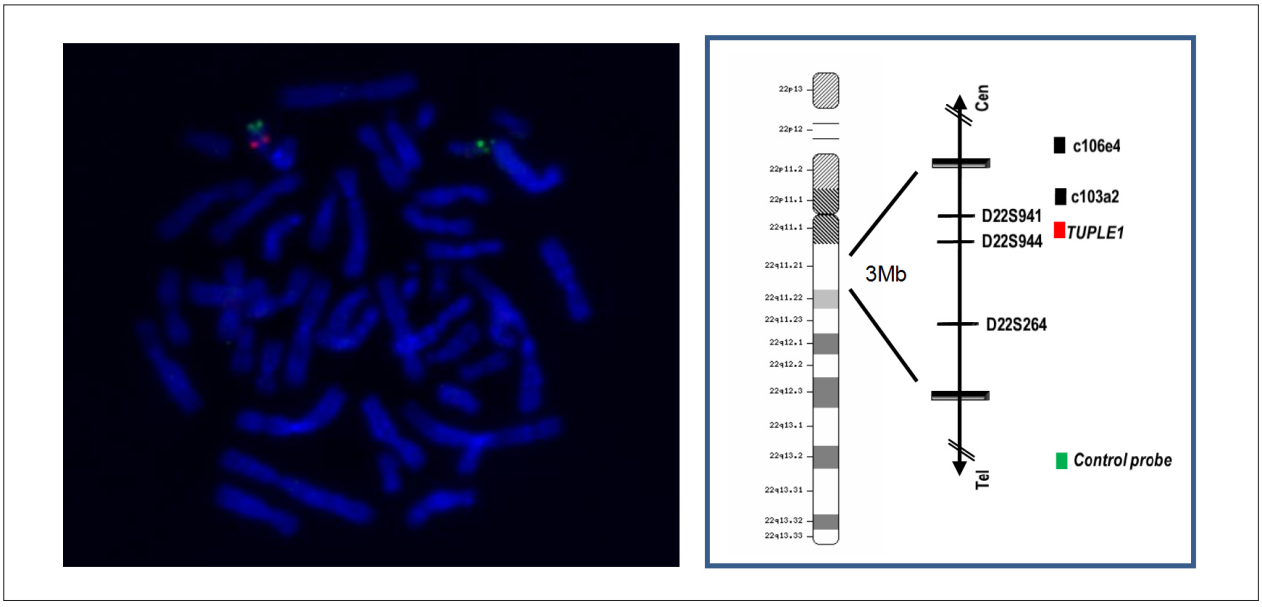

Figure 1 - (a) Metaphase of a patient with the 22q11.2 deletion, submitted to the FISH technique. The red signal indicates the 22q11.2 region and the green signal the terminal chromosome 22 region, used as control. The arrow indicates the deleted chromosome 22, showing the presence of the control region only. (b) Schematic representation of chromosome 22, showing the usually deleted 3Mb region, the polymorphic DNA markers and the FISH probes used in this study.

arch, a heart defect present in 50% of the cases of 22q11.2 deletion[13]. However, as the karyotype revealed the presence of an isodicentric marker derived from chromosome 22, the final diagnosis was partial tetrasomy of chromosome 22 or cat eye syndrome (CES), a rare malformation syndrome of which diagnosis is based on the presence of an extra marker chromosome, derived from chromosome 22[14]. In this case, the FISH technique also proved to be efficient in refining the cytogenetic diagnosis.

The frequency of the 22q11.2 deletion associated with the 22q11.2 deletion syndrome has not been well established in the literature and may vary, among other factors, according to the sample studied and the technique used for its detection. Molecular analysis including DNA markers and FISH shows that the deletion is present in about 80-90% of the syndrome cases[2,15,16]. In our study, the 22q11.2 deletion was found in 25% of the sample, a frequency that is lower than the one reported in the literature.

A factor that might explain this low frequency of the deletion is the great phenotypic variability observed in the patients of this study, as our sample comprised from patients with isolated heart defect to patients with the complete clinical picture of the syndrome. According to the literature, the frequency of the 22q11.2 deletion among patients with isolated conotruncal heart defect is about 29%[17,18], much lower than the deletion frequency among patients with a heart defect associated with other clinical signs (80-90%)[2,15,16]. In our series, none of the six patients with isolated heart defect presented the 22q11.2 deletion, a finding that corroborates the idea that the deletion is more frequent in patients who present, in addition to the heart defect, other associated phenotypic signs[17,18].

All the deletions were found in patients with the clinical spectrum of the syndrome, both in patients with a heart defect and in patients without it. These data show that patients that present facial phenotypic characteristics of the syndrome, even without heart malformations, should be investigated for the presence of the deletion.

The patients' age may also have influenced the frequency of the deletion, as the age range of the selected patients varied greatly and the phenotype is not always so evident in very young children.

Several individuals (15/22), although bearers of the clinical signs of the syndrome, did not present a detectable deletion and, even though the 22q11.2 deletion is the most likely etiology, other causes may be responsible for this phenotype. Among them, there are mutations in the genes located in this region (22q11.2) and in genes from other regions related to congenital conotruncal heart defects, such as 8p23.1[19] and 10p13[20].

Concerning the use of polymorphic DNA markers, our results led us to the conclusion that the ideal procedure is to study the proband and his (her) two parents, although if the patient is found to be heterozygous, this result is enough to indicate absence of the deletion, regardless of the parents' molecular study. Thus, a molecular evaluation can be performed in children with the 22q11.2 deletion syndrome phenotype as a first triage test to detect the 22q deletion, before using FISH. Moreover, when it is impossible to collect peripheral blood from the patients, molecular assays can be performed with DNA extracted from other tissues.

In this study, we used three DNA markers located in the region that is deleted in about 98% of the patients with the syndrome phenotype, which increases the success rate in the investigation of the deletion. However, these markers may not detect atypical deletions, i.e., deletions of segments which are outside the typically deleted region and which occur at a frequency of 2% in patients with the syndrome phenotype[4].

The 22q11.2 deletion was not present in any of the parents of the children with the deletion, thus characterizing all the deletions detected in the probands as sporadic.

Conclusions

The data obtained in this study suggest that the 22q11.2 deletion is more frequent in patients with the clinical picture of the 22q11.2 deletion syndrome than in patients with isolated conotruncal heart defect. Regarding the detection methodologies used, the FISH technique is more accurate for detecting the deletion than the DNA marker technique. The FISH technique allows investigating the presence of chromosome alterations in the parents of children who carry the deletion, which determines the couple's reproductive risk. Investigating the presence of the deletion and its correlation with the patients' clinical data can help the cardiologist to provide a better follow-up for the patients.

Potential Conflict of Interest

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

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Study Association

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