

Molecular Screening for 22Q11.2 Deletion Syndrome in Patients With Congenital Heart Disease

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Abstract Few studies have investigated the prevalence of 22q11.2 deletion syndrome (22q11.2DS) among patients with isolated heart defects or nonconotruncal heart defects. Polymerase chain reaction (PCR) followed by length polymorphism restriction fragment analysis (RFLP) is useful for low-cost molecular diagnosis and screening. This cross-sectional study included 392 patients with congenital heart disease, described clinical features, and performed PCR–RFLP for analysis of polymorphism in three loci with a high heterozygosity rate located in the typically deleted region of 1.5 megabases. Heterozygosity excluded 22q11.2DS. Patients with homozygosity for the three markers underwent multiplex ligation-dependent probe amplification (MLPA) and fluorescence in situ hybridization (FISH) for the final diagnosis, estimating the prevalence of 22q11.2DS. The use of PCR–RFLP excluded

22q11.2DS in 81.6 % ($n = 320$) of 392 patients. Of the remaining 72 patients, 65 underwent MLPA, showing 22q11.2DS in five cases (prevalence, 1.27 %). Four of these five patients underwent FISH, confirming the MLPA results. All five patients with the deletion had heart diseases commonly found with 22q11.2DS (interrupted aortic arch, persistent truncus arteriosus, tetralogy of Fallot, and ventricular septal defect plus atrial septal defect). Two patients had congenital extracardiac anomaly (one with arched palate and micrognathia and one with hypertelorism). Three patients reported recurrent respiratory infections, and one patient reported hypocalcemia. All were underweight or short in stature for their age. This study contributed to showing the prevalence of 22q11.2DS in patients with any congenital heart disease, with or without other features of the syndrome. Patients with 22q11.2DS may not have all the major features of the syndrome, and those that are found may be due to the heart defect.

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Introduction

The 22q11.2 deletion syndrome (22q11.2DS), with an incidence of 1 in every 4,000–5,000 live births [30], is the second leading chromosomal cause of congenital heart disease [16]. Congenital heart diseases, mainly conotruncal, are related to this syndrome in 75 % of cases [29, 37].

The 22q11.2 region contains long stretches of repeated sequences clustered together and known as low-copy repeats (LCRs) sharing more than 95 % of identity. The deletion occurs by nonallelic homologous recombination between these LCRs. Most patients (90 %) had deletions in

the same region of about 3 megabases (Mb) called the DiGeorge syndrome-critical region, which contains about 40 genes [5, 32].

Fluorescence in situ hybridization (FISH), the gold standard test to determine 22q11.2DS, detects more than 95 % of cases but is very expensive, time consuming, and not widely available, precluding its use for widespread screening in settings with limited resources [7, 8]. Recently, studies have shown that polymerase chain reaction (PCR) assays with analysis of restriction fragment length polymorphism (RFLP) based on homozygosity at consecutive markers in the DiGeorge chromosomal region can be useful in screening for microdeletions with less cost as well as high sensitivity and specificity [8, 11, 14, 26] but that PCR–RFLP should be complemented by FISH [8, 26] or multiplex ligation-dependent probe amplification (MLPA) for the final diagnosis.

The MLPA method is reliable, fast, and economic, with 95–99 % sensitivity and 97–99 % specificity [10, 36]. The advantages of PCR over FISH are agility (because FISH requires cell culture), facility, low cost per sample, small number of deoxyribonucleic acid (DNA) requirements, possibility of using samples with longer storage, and greater sensitivity in screening for 22q11.2 deletion [19].

The prevalence of 22q11.2DS may be greater than known due to the existence of cases with mild or atypical manifestations [24] or nonconotruncal heart defects [38]. Few studies investigating the prevalence of 22q11.2DS have included patients with isolated heart defects who show no other features of the deletion. Early identification of the syndrome allows searching for associated anomalies, provision of genetic counseling, and facilitation of appropriate multidisciplinary management [17].

This study aimed to estimate the prevalence and clinical features of 22q11.2DS in a sample of patients who had congenital heart disease, with or without manifestations of 22q11.2DS, including patients with conotruncal and other congenital heart disease, using PCR–RFLP to exclude the deletion and performing MLPA and FISH in cases for which it was not possible to exclude deletion by PCR–RFLP.

Materials and Methods

This cross-sectional study included 392 patients with congenital heart disease followed at the Pediatric Cardiology Division of Instituto de Cardiologia/Fundação Universitária de Cardiologia (IC–FUC), Porto Alegre, Brazil. These patients were selected by random sampling from January 2007 to May 2008. Patent ductus arteriosus in children younger than 3 months and patent foramen ovale were not considered congenital heart defects. We excluded patients with Down syndrome, Edward's syndrome, and Patau syndrome, which are strongly associated with congenital heart

disease [25]. The study also excluded patients who received blood transfusions in the 4 months before blood collection to avoid contamination of the donor DNA [35].

All the patients or their guardians signed the informed consent before their inclusion in the study. The study protocol was approved by the institutional Ethics Committee and performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

None of the patients who fulfilled the inclusion criteria refused to participate. The patients, their parents, or both were interviewed to obtain information about symptoms, serum calcium, psychomotor development, presence of other congenital anomalies, and family history.

Clinical examination and echocardiography with two-dimensional image and color Doppler were performed. Anthropometric measurements were obtained for verification of percentiles of weight for age and of height for age according to tables from the National Center for Health Statistics/National Center for Chronic Disease Prevention and Health Promotion–2000 [20]. The patients' charts were reviewed to obtain data about heart disease.

DNA Extraction for Genetic Evaluation

Venipuncture was used to collect 5 ml of blood from each patient. The Lahiri and Nurnberger [21] method was used for DNA extraction. In cases for which new blood collection was necessary, DNA extraction was performed with Pure-Link Genomic DNA Kits (Invitrogen, São Paulo, Brazil).

PCR–RFLP

To exclude the presence of 22q11.2 microdeletion, PCR–RFLP was performed, with analysis of the polymorphism in rs4819523, rs5748411, and rs4680 loci, which lie in the affected region of typical 1.5 Mb deletion in 22q11.2DS [14]. Reactions were performed in the TC-412 thermal cycler (Techne, Stone, UK) in a final volume of 25 μ l using 10 pmol of each primer, 1 \times PCR buffer, 2 mmol/l of MgCl₂, 0.2 mmol/l of dNTP, and 1 U of Taq DNA polymerase.

All the reagents for PCR were purchased from Invitrogen (São Paulo, Brazil). The quantity of DNA used in each reaction was approximately 200 ng for amplification of loci rs4819523 and rs4680 and approximately 10 ng for amplification of locus rs5748411. The primers used for specific PCR, amplification conditions, and amplicon sizes are shown in Table 1. We designed these primers.

To prove effective amplification of different fragments, PCR products were analyzed by electrophoresis in 1 % agarose gel stained with GelRed dye (Biotium, Hayward, CA, USA). Images were documented in EC3 Imaging Systems (UVP, Upland, CA, USA).

Table 1 Primers used for specific PCR–RFLP, amplicon sizes, and amplification conditions^a

Locus	Primers	Amplicon size (bp)	Amplification conditions
rs5748411	ATCAGCCCACAGAGCAGACC/ GCATAGAGAAAAGCAACTCC	202	50 cycles (95 °C for 40 s; 62 °C for 40 s; 72 °C for 40 s)
rs4819523	ACAGTGAGGGGACATCAAGG/ GGAAAGCTCATGGGTGTCTG	304	50 cycles (95 °C for 40 s; 58 °C for 40 s; 72 °C for 40 s)
rs4680	GTGATTCAGGAGCACCAGCC/ GCCCTTTTCCAGGTCTGAC	217	50 cycles (95 °C for 40 s; 58 °C for 40 s; 72 °C for 40 s)

PCR–RFLP polymerase chain reaction followed by length polymorphism restriction fragment analysis, *bp* base pairs

^a Initial melting step at 95 °C for 5 min and final extension step at 72 °C for 7 min were used in all reactions

The amplification products were cleaved by the following restriction enzymes according to the manufacturer's recommendations (Fermentas, Vilnius, Vilnius County, Lithuania): *Hae*III (for rs4819523 locus) with cleavage products of 156, 75, 38, and 35 base pairs (bp) in the presence of the cleavage site in the polymorphic locus or 156, 110 and 38 bp in the absence of the cleavage site in the polymorphic locus; *Bcl*II (for rs4680 locus) with cleavage products of 102 and 115 bp; and *Bsr*I (for rs5748411 locus) with cleavage products of 64 and 138 bp. The cleavage products were analyzed in 0.75 % agarose with 1.65 % Synergel (BioAmerica, Miami, FL, USA), equivalent to 4 % agarose gel. Samples were stained with GelRed dye (Biotium). The images were documented in EC3 Imaging Systems.

The detection of heterozygosity in the first marker was sufficient to exclude the presence of 22q11.2 microdeletion. In cases of homozygosity, PCR–RFLP was performed for other loci. When homozygosity was detected in the three analyzed loci, the sample was subsequently analyzed by the MLPA method.

MLPA

Samples with homozygosity for the three consecutive PCR–RFLP markers were analyzed by MLPA using SALSA MLPA kit P023-B2 DiGeorge syndrome/VCF (MRC-Holland, Amsterdam, Netherlands) according to the manufacturer's recommendations. Amplified products were separated by capillary gel electrophoresis in the ABI PRISM 3130XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) and analyzed using Coffalyser V9.4 Software (MRC-Holland). All analyzes were performed in duplicate.

FISH

Patients with del22q11.2 detected by MLPA together with their parents were subjected to FISH analysis. Metaphase chromosome preparations were obtained from phytohemagglutinin-stimulated lymphocyte cultures according to standard procedures. The FISH procedure was performed on

metaphase spreads from peripheral blood lymphocyte cultures. The DiGeorge/VCFs TUPLE1 probe (Cytocell, Cambridge, UK) was used according to the standard manufacturer's protocol. In each case, 30 metaphase cells and 100 interphase cells were examined. Hybridizations were analyzed with an epifluorescence microscope, and images were captured with a CCD camera. We considered a chromosome region deleted when the FISH signal from the corresponding probe was absent on one of the homolog chromosomes.

Statistical Analysis

The prevalence of 22q11.2DS approximating 1.47 % in patients with congenital heart disease, as observed by Botto et al. [2], was considered in the calculation of the sample size, with a 95 % confidence interval (CI) of 0.3–2.7 %. A total of 392 patients were estimated.

The characteristics of the sample were analyzed by mean or median for quantitative variables and by proportions for qualitative variables. To determine prevalence, 95 % CIs were calculated. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) (SPSS, Chicago, IL, USA).

Results

The age at the time of blood collection for research on 22q11.2DS ranged from 14 days to 66 years (median 10.9 months). The median age at diagnosis of heart disease was 1 month. Of the 392 patients, 15 (3.8 %) had an echocardiographic diagnosis in the prenatal period. Extra-cardiac congenital anomalies, both isolated or as part of syndromes, were found in 114 patients (29.1 %). The specific congenital heart lesions are described in Table 2.

PCR–RFLP

The sequence of PCR–RFLP tests, their results (homo- or heterozygosity for each locus), and analysis of

Table 2 Distribution of the 392 patients regarding the type of congenital heart disease

Heart disease	No. of patients (%)
Tetralogy of Fallot	51 (13)
VSD with or without bicuspid aortic valve, PDA, or ASD	50 (12.7)
Obstructive lesions of RVOT with or without ASD	47 (12.0)
ASD	34 (8.7)
Lesions of LVOT	34 (8.7)
CoAo with or without other mild defect	33 (8.4)
TGA	23 (5.9)
Double-outlet or double-inlet ventricle	22 (5.6)
VSD and obstructive lesions of RVOT or LVOT	19 (4.9)
ECD	13 (3.3)
Ebstein's anomaly	12 (3.1)
PDA	8 (2)
Absence of an atrioventricular connection	8 (2)
Mitral valvulopathy	6 (1.5)
Congenitally corrected TGA	6 (1.5)
Pulmonary atresia with VSD	6 (1.5)
Pulmonary atresia with intact ventricular septum	5 (1.3)
Anomalous pulmonary venous return	5 (1.3)
Persistent truncus arteriosus	2 (0.5)
Cor triatriatum	2 (0.5)
Obstructive lesions of RVOT and LVOT	1 (0.3)
Interrupted aortic arch with bicuspid aortic valve and VSD	1 (0.3)
Hemitruncus arteriosus	1 (0.3)
Pulmonary valve regurgitation	1 (0.3)
ECD and double aortic arch	1 (0.3)
Double aortic arch with absent pulmonary valve	1 (0.3)

VSD ventricular septal defect, PDA patent ductus arteriosus, ASD atrial septal defect, RVOT right ventricle outlet tract, LVOT left ventricle outlet tract, CoAo coarctation of the aorta, TGA transposition of the great arteries, ECD endocardial cushion defect

representative PCR–RFLP images are shown in Fig. 1. Analysis of the rs4819523 locus in the 392 patients showed homozygosity for the allele with the cleavage site in 77 patients (19.6 %), homozygosity for the allele without the cleavage site in 118 patients (30.1 %), and heterozygosity in 197 patients (50.3 %), excluding 22q11.2 microdeletion in these 197 patients.

For 195 patients with homozygosity at the rs4819523 locus, analysis of the rs4680 locus was performed, showing homozygosity for the allele with the cleavage site in 44 patients (22.6 %), homozygosity for the allele without the cleavage site in 82 patients (42.1 %), and heterozygosity in 69 patients (35.4 %), excluding the microdeletion in these 69 patients.

For 126 patients with homozygosity at the rs4680 locus, analysis of the rs5748411 locus was performed, showing

homozygosity for the allele with the cleavage site in 51 patients (40.5 %), homozygosity for the allele without the cleavage site in 21 patients (16.7 %), and heterozygosity in 54 patients (42.9 %), excluding the presence of microdeletion in these 54 patients. Thus, analysis of polymorphism of three loci affected in the 1.5-Mb region with typical deletion (rs4819523, rs4680, and rs5748411) by PCR–RFLP allowed 22q11.2 microdeletion to be ruled out for 320 patients (81.6 %).

MLPA

Of the 72 cases (18.4 %) for which it was not possible to rule out the presence of microdeletion 22q11.2 using PCR–RFLP, 65 were subjected to MLPA, and 22q11.2 microdeletion was found in five patients. The remaining seven patients (who were not subjected to MLPA) were invited to collect blood to improve the DNA sample quality for MLPA, but two of the patients did not accept, and five were not found after an exhaustive search. The prevalence of 22q11.2DS was 1.27 % (5 of 392 patients; 95 % CI, 0.16–2.38 %) (Fig. 1).

FISH

Four of the five patients with 22q11.2 microdeletion detected by MLPA were subjected to FISH. This analysis showed a signal only on the normal homologue chromosome, indicating deletion of band 22q11.2, confirming MLPA results. The fifth patient died of coagulopathy and cardiogenic shock after heart surgery before collection of blood for FISH (Fig. 1).

In one case, FISH was performed for both parents. In two cases, blood samples were available only from the mother. In one case, the parents did not agree to the collection of blood. Metaphase FISH examinations from the parents showed normal results, with no indication of rearrangement involving the chromosome 22q11.2 critical region.

Clinical Features

The clinical features of patients who had confirmed 22q11.2DS are described in Table 3.

Discussion

The current study, conducted in a referral center, evaluated a large number of outpatients with a proportional sample of congenital heart disease using an inexpensive diagnostic approach and found a 22q11.2DS prevalence of 1.27 % (5 of 392 patients; 95 % CI, 0.16–2.38 %). The prevalence would be 1.29 % (5 of 385 patients; 95 % CI,

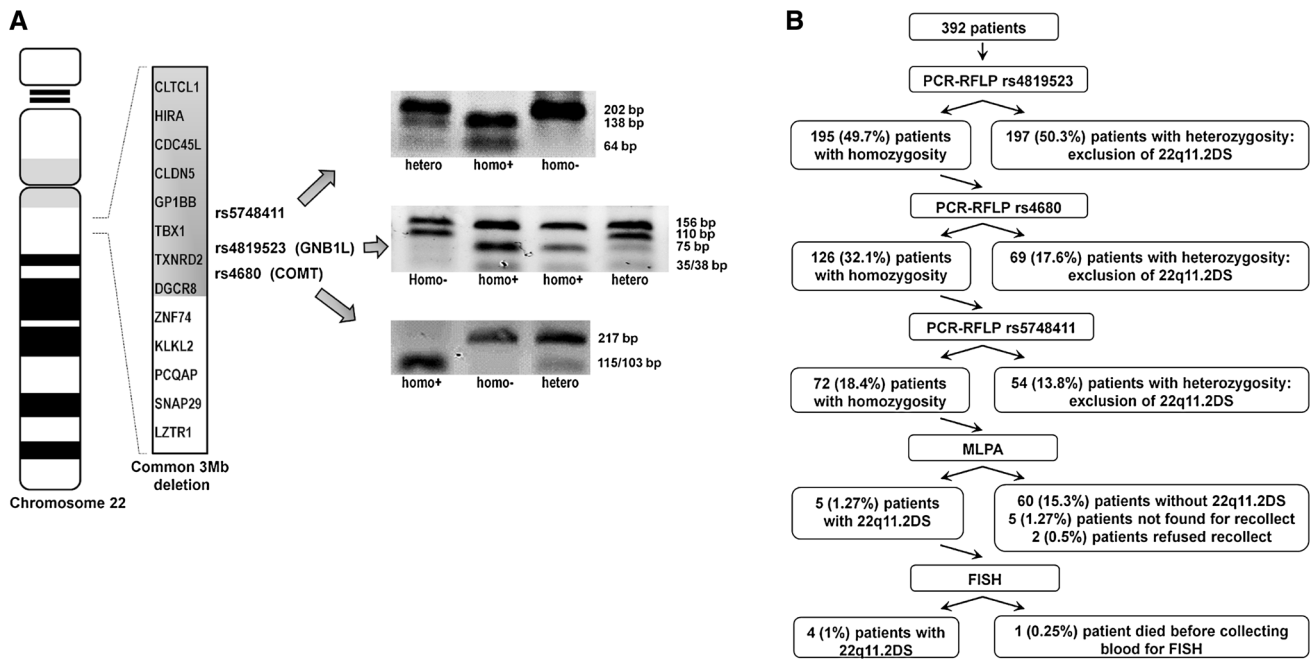


Fig. 1 a Graphic representation of the genomic loci with representative PCR–RFLP image analysis for each locus (rs5748411, rs4819523, and rs4680) showing amplification products and cleavage products, with homozygosity for the presence of a cleavage site in the polymorphic locus (homo+) and with homozygosity for the absence

of a cleavage site (homo–) and heterozygosity (hetero). **b** Sequence of tests (PCR–RFLP, MLPA, and FISH) and results. *PCR–RFLP* polymerase chain reaction followed by length polymorphism restriction fragment analysis, *MLPA* multiplex ligation-dependent probe amplification, *FISH* fluorescence in situ hybridization

Table 3 Clinical features of patients with 22q11.2DS

Patient	Age	Gender	Heart disease	Features	Observations
1	18 years	F	Interrupted aortic arch with bicuspid aortic valve and VSD	Mild neurodevelopment delay, hearing loss, recurrent respiratory infections, hypocalcemia, low weight and height for age	DiGeorge syndrome previously diagnosed without supporting documents
2	12 years	F	Persistent truncus arteriosus (type 1)	Hearing loss, seizures, recurrent respiratory infections, low weight for age in the past, low height for age	Mother and father with negative MLPA and FISH
3	21 years	F	Tetralogy of Fallot with right aortic arch	Arched palate and micrognathia, mild neurodevelopment delay, epilepsy, low weight for age in the past	Mother with negative MLPA and FISH
4	4 years	F	VSD and ASD	Mild neurodevelopment delay, recurrent respiratory infections, low weight and low height for age	Mother with negative MLPA and FISH
5	11 months	M	Tetralogy of Fallot	Hypertelorism and low weight for age	Death of the patient before collection of blood for FISH

F female, *VSD* ventricular septal defect, *MLPA* multiplex ligation dependent probe amplification, *FISH* fluorescence in situ hybridization, *ASD* atrial septal defect, *M* male

0.16–2.42 %) if the seven patients not subjected to MLPA were excluded or 3 % (12 of 392 patients; 95 % CI, 1.31–4.69 %) if all seven of these patients were carriers of the 22q11.2 microdeletion.

This study included patients with all types of congenital heart disease, not only conotruncal disease, with or without extracardiac manifestations of 22q11.2DS. Recent data from the same region of the country using FISH showed a

22q11DS prevalence of 2 % in 198 patients with congenital heart disease who were hospitalized in a pediatric intensive care unit (i.e., patients with more severe heart disease than those evaluated in the current study) [28]. A study in India detected SD22q11.2 in 5.7 % of 105 patients who had congenital heart disease without extracardiac malformations [12]. Another study, in the United States, detected 22q11.2DS in 1.47 % of 2,396 patients with major congenital heart disease [2].

In 2003, Pereira et al. [26] conducted a population-based study in São Paulo, Brazil and concluded that the specificity of the test using three markers was 98.3 %. Adding a fourth marker did not achieve a significant increase in specificity. The results showed that PCR–RFLP can be a very good test for exclusion.

We used three DNA markers located in the region of 1.5 Mb, which is deleted in the majority of patients with 22q11.2DS [5, 32]. This approach would not detect only the rare cases of atypical deletions [27]. The PCR method has the disadvantage of failing to detect mosaicism [9], but this is a rare find in 22q11.2DS [18]. It was possible to exclude the presence of 22q11.2 microdeletion by PCR–RFLP in 81.6 % of the patients, leaving few patients for the use of MLPA. As a confirmatory test, this approach was fast, accurate, and cost effective, showing concordance with FISH findings in studies investigating 62 patients [36], 30 patients [10], and 51 patients [33] with 22q11.2DS.

Previous studies demonstrated that the spectrum of clinical features in 22q11.2DS is very varied [24] and that individuals with similar molecular defects show clinical variability, which seems unrelated to the size of the deleted region [1]. More than 180 clinical features in 22q11.2DS have been described [29, 37]. Immunodeficiency occurs in 40–93 %, hypoparathyroidism in 40–60 % [23], and palatal abnormalities in 69 % [4] of patients with 22q11.2DS. Neurologic and psychiatric disorders also may occur [6, 13].

Congenital heart disease may be nonconotruncal [29, 38]. According to reports, hypoparathyroidism may manifest only after cardiac surgery [31]. In our study, patients in the 22q11.2DS group showed varied features of the syndrome. Only one of the five patients had previous suspicion of the deletion. This patient had interrupted aortic arch, a heart defect in which 50 % of the patients have the deletion [2]. All five patients had heart diseases commonly found with this syndrome [29], but they did not have all the major features of the 22q11.2DS. At physical examination, only two had congenital extracardiac anomalies, and these abnormalities were not pathognomonic features of the 22q11.2DS. No cases of hypoparathyroidism were found, but asymptomatic patients may not have been identified because parathyroid hormone was not systematically evaluated.

The reported respiratory infections could have been due to heart disease or primary or secondary immunodeficiency due to another cause. Poor weight or height gain could have been due to heart disease and its therapeutic interventions, not necessarily 22q11.2 microdeletion. However, according to Brauner et al. [3], short stature may be due to growth hormone deficiency in patients with 22q11.2DS. Developmental delay also could be due to heart disease or its interventions, as suggested by other authors [22]. However, Van Aken et al. [34] showed motor deficits and a negative effect on the intelligence quotient without the effect of congenital heart disease in the group of children with 22q11.2DS compared with the control group. Nonetheless, in our study, seizures were reported by 11.5 % of the patients, which is much higher than the incidence in the general population (1–3 %) [15].

In conclusion, PCR–RFLP for screening of 22q11.2DS in patients who have any congenital heart disease, with or without other features of the syndrome, allows the presence of deletion in most patients to be excluded at low cost, and the final diagnosis can be established by a complementary method such as FISH or MLPA. Patients with 22q11.2DS may not have all the major features of the syndrome, and those that are found may be due to the heart defect or other causes, delaying the early diagnosis of other abnormalities and genetic counseling. This study contributed to showing a more real prevalence of 22q11.2DS in patients with congenital heart disease because few studies have investigated the prevalence of 22q11.2DS in patients who have any heart defects, not only conotruncal defects, with or without other features of the syndrome.

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Conflict of interest The authors declare that they have no conflict of interest.

References

- Amati F, Conti E, Novelli A et al (1999) Atypical deletions suggest five 22q11.2 critical regions related to the DiGeorge/velo-cardio-facial syndrome. *Eur J Hum Genet* 7:903–909
- Botto LD, May K, Fernhoff PM et al (2003) A population-based study of the 22q11.2 deletion: phenotype, incidence, and contribution to major birth defects in the population. *Pediatrics* 112:101–107
- Brauner R, Le Harivel de Gonville A, Kindermans C et al (2003) Parathyroid function and growth in 22q11.2 deletion syndrome. *J Pediatr* 142:504–508

4. Chegar BE, Tatum SA, Marrinan E, Shprintzen RJ (2006) Upper airway asymmetry in velo-cardio-facial syndrome. *Int J Pediatr Otorhinolaryngol* 70:1375–1381
5. Chen YF, Kou PL, Tsai SJ et al (2006) Computational analysis and refinement of sequence structure on chromosome 22q11.2 region: application to the development of quantitative real-time PCR assay for clinical diagnosis. *Genomics* 87:290–297
6. Debbané M, Glaser B, David MK, Feinstein C, Eliez S (2006) Psychotic symptoms in children and adolescents with 22q11.2 deletion syndrome: neuropsychological and behavioral implications. *Schizophr Res* 84:187–193
7. Driscoll DA (1994) Genetic basis of DiGeorge and velocardio-facial syndromes. *Curr Opin Pediatr* 6:702–706
8. Driscoll DA, Emanuel BS, Mitchell LE, Budarf ML (1997) PCR assay for screening patients at risk for 22q11.2 deletion. *Genet Test* 1:109–113
9. Eiris-Puñal J, Iglesias-Meleiro JM, Blanco-Barca MO et al (2003) Phenotypic variability of deletion 22q11.2: an analysis of 16 observations with special emphasis on the neurological manifestations. *Rev Neurol* 37:601–607 (in Spanish)
10. Fernández L, Lapunzina P, Arjona D et al (2005) Comparative study of three diagnostic approaches (FISH, STRs and MLPA) in 30 patients with 22q11.2 deletion syndrome. *Clin Genet* 68:373–378
11. Funke BH, Brown AC, Ramoni MF et al (2007) A novel, single-nucleotide polymorphism-based assay to detect 22q11 deletions. *Genet Test* 11:91–100
12. Gawde H, Patel ZM, Khatkhatey MI et al (2006) Chromosome 22 microdeletion by FISH in isolated congenital heart disease. *Indian J Pediatr* 73:885–888
13. Gerdes M, Solot C, Wang PP et al (1999) Cognitive and behavior profile of preschool children with chromosome 22q11.2 deletion. *Am J Med Genet* 85:127–133
14. Gioli-Pereira L, Pereira AC, Mesquita SM, Lopes AA, Krieger JE (2006) PCR screening for 22q11.2 microdeletion: development of a new cost-effective diagnostic tool. *Clin Chim Acta* 369:78–81
15. Gitai DL, Romey-Pereira RN, Gitai LL, Leite JP, Garcia-Cairasco N, Paco-Larson ML (2008) Genes and epilepsy I: epilepsy and genetic alterations. *Rev Assoc Med Bras* 54:272–278 (in Portuguese)
16. Goodship J, Cross I, LiLing J, Wren C (1998) A population study of chromosome 22q11 deletions in infancy. *Arch Dis Child* 79:348–351
17. Greenhalgh KL, Aligianis IA, Bromilow G et al (2003) 22q11 Deletion: a multisystem disorder requiring multidisciplinary input. *Arch Dis Child* 88:523–524
18. Halder A, Jain M, Kabra M, Gupta N (2008) Mosaic 22q11.2 microdeletion syndrome: diagnosis and clinical manifestations of two cases. *Mol Cytogenet* 1:18
19. Kariyazono H, Ohno T, Ihara K et al (2001) Rapid detection of the 22q11.2 deletion with quantitative real-time PCR. *Mol Cell Probes* 15:71–73
20. Kuczumarski RJ, Ogden CL, Guo SS et al (2002) 2000 CDC growth charts for the United States: methods and development. *Vital Health Stat* 11:1–190
21. Lahiri DK, Nurnberger JI Jr (1991) A rapid nonenzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res* 19:5444
22. Maharasingam M, Ostman-Smith I, Pike MG (2003) A cohort study of neurodevelopmental outcome in children with DiGeorge syndrome following cardiac surgery. *Arch Dis Child* 88:61–64
23. McDonald-McGinn DM, Kirschner R, Goldmuntz E et al (1999) The Philadelphia story: the 22q11.2 deletion: report on 250 patients. *Genet Couns* 10:11–24
24. Moreno Izco F, Zuazo Zamalloa E, González Alvaredo S, Bereciartu Irastorza P (2009) 22q11 Deletion syndrome: an expanding phenotype. *Neurologia* 24:69–71 (in Spanish)
25. Payne RM, Johnson MC, Grant JW, Strauss AW (1995) Toward a molecular understanding of congenital heart disease. *Circulation* 91:494–504
26. Pereira AC, Corrêa RF, Mota GF, Kim CA, Mesquita SF, Krieger JE (2003) High-specificity PCR screening for 22q11.2 microdeletion in three different ethnic groups. *Braz J Med Biol Res* 36:1359–1365
27. Rauch A, Zink S, Zweier C et al (2005) Systematic assessment of atypical deletions reveals genotype–phenotype correlation in 22q11.2. *J Med Genet* 42:871–876
28. Rosa RF, Pilla CB, Pereira VL et al (2008) 22q11.2 Deletion syndrome in patients admitted to a cardiac pediatric intensive care unit in Brazil. *Am J Med Genet A* 146A:1655–1661
29. Ryan AK, Goodship JA, Wilson DI et al (1997) Spectrum of clinical features associated with interstitial chromosome 22q11 deletions: a European collaborative study. *J Med Genet* 34:798–804
30. Scambler PJ (2000) The 22q11 deletion syndromes. *Hum Mol Genet* 9:2421–2426
31. Schaan BD, Huber J, Leite JC, Kiss A (2006) Cardiac surgery unmasks latent hypoparathyroidism in a child with the 22q11.2 deletion syndrome. *J Pediatr Endocrinol Metab* 19:943–946
32. Shaikh TH, O'Connor RJ, Pierpont ME et al (2007) Low copy repeats mediate distal chromosome 22q11.2 deletions: sequence analysis predicts breakpoint mechanisms. *Genome Res* 17:482–491
33. Stachon AC, Baskin B, Smith AC et al (2007) Molecular diagnosis of 22q11.2 deletion and duplication by multiplex ligation dependent probe amplification. *Am J Med Genet A* 143A:2924–2930
34. Van Aken K, De Smedt B, Van Roie A et al (2007) Motor development in school-aged children with 22q11 deletion (velo-cardiofacial/DiGeorge syndrome). *Dev Med Child Neurol* 49:210–213
35. Vervoordeldonk SF, Doumaid K, Remmerswaal EB et al (1998) Long-term detection of microchimaerism in peripheral blood after pretransplantation blood transfusion. *Br J Haematol* 102:1004–1009
36. Vorstman JA, Jalali GR, Rappaport EF, Hacker AM, Scott C, Emanuel BS (2006) MLPA: a rapid, reliable, and sensitive method for detection and analysis of abnormalities of 22q. *Hum Mutat* 27:814–821
37. Widdershoven JC, Beemer FA, Kon M, Dejonckere PH, Mink van der Molen AB (2008) Possible mechanisms and gene involvement in speech problems in the 22q11.2 deletion syndrome. *J Plast Reconstr Aesthet Surg* 61:1016–1023
38. Wozniak A, Wolnik-Brzozowska D, Wisniewska M et al (2010) Frequency of 22q11.2 microdeletion in children with congenital heart defects in western Poland. *BMC Pediatr* 10:88