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Short Communication

Fluorescence *in situ* hybridization (FISH) screening for the 22q11.2 deletion in patients with clinical features of velocardiofacial syndrome but without cardiac anomalies

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Abstract

The velocardiofacial syndrome (VCFS), a condition associated with 22q11.2 deletions, is characterized by a typical facies, palatal anomalies, learning disabilities, behavioral disturbances and cardiac defects. We investigated the frequency of these chromosomal deletions in 16 individuals with VCFS features who presented no cardiac anomalies, one of the main characteristics of VCFS. Fluorescent *in situ* hybridization (FISH) with the N25 (D22S75; 22q11.2) probe revealed deletions in ten individuals (62%). Therefore, even in the absence of cardiac anomalies testing for the 22q11.2 microdeletions in individuals showing other clinical features of this syndrome is recommended.

Key words: velocardiofacial syndrome, 22q11.2 deletion.

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Chromosome 22q11.2 deletions are associated with a wide spectrum of phenotypes, including velocardiofacial syndrome (VCFS), DiGeorge syndrome (DGS), conotruncal anomalies and sporadic or familial cardiac defects (Lipson *et al*., 1991; Scambler 2000). These deletions encompass 1.5 to 3 Mb (Lindsay *et al*., 1995a,b; Adeyinka *et al*., 2004) and are most often the result of a *de novo* event, although 5-10% are inherited (Sandrin-Garcia *et al*., 2002).

Velocardiofacial syndrome (VCFS) is characterized by a complex of clinical anomalies: typical facies, velopharyngeal insufficiency (VPI) or cleft palate, learning disabilities, behavioral disturbances and cardiac anomalies (Shprintzen, 1990; Lipson *et al*., 1991). The typical facies includes a prominent nose with squared nasal root and narrow alar base, relative deficiency of the malar area, vertical maxillary excess, retruded mandible, narrow palpebral fissures and minor ear anomalies. Palatal anomalies, learning disabilities and typical facies are present in all patients. Cardiac defects are found in 84% of the patients and are the main cause of morbidity and mortality (Shprintzen *et al*., 1981). Other less frequent features are psychiatric disorders, short stature and hyperextensibility of the digits. The estimated incidence of the syndrome is 1:4000 live births (Devriendt *et al*., 1998).

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Cytogenetic studies using high-resolution chromosome banding have detected 22q11.2 deletions in approximately 20% of individuals with VCFS (Driscoll *et al*., 1992), whereas fluorescent in situ hybridization (FISH) analysis has demonstrated 22q11.2 microdeletions in the majority of these patients (Lindsay *et al.*, 1995 a,b). A clear correlation between the extension of the deletions and the clinical variability has not become evident (Carlson *et al*., 1997 a,b; Edelman *et al*. 1999; Digilio *et al*., 2003).

Some studies investigated the frequency of cardiac anomalies among carriers of 22q11.2 deletions. In a European collaborative study of 558 individuals with 22q11 deletions (Ryan *et al*., 1997), 70% of the 545 individuals evaluated for cardiac anomalies had a significant cardiac pathology, such as Fallot's tetralogy, ventricular septal defect, interrupted aortic arch, pulmonary atresia/ventricular septal defect or truncus arteriosus. Similar frequencies

were observed by McDonald-McGinn *et al*. (1999) and Kitsiou-Tzeli *et al*. (2004). However, Bassett *et al*. (2005) reported cardiac anomalies in 25.8% of 78 adults carrying 22q11 deletions.

To investigate the association of 22q11.2 deletions with cardiac anomalies, we searched for 22q11.2 deletions in individuals with clinical features of VCFS who did not present with cardiac anomalies.

After the approval by the Committee of Ethics in Research of the Ribeirão Preto Medical School University of São Paulo and obtaining written informed consent from the individuals or their legal guardians, we selected a group of 16 individuals (6 females, 10 males) among those referred to the Hospital de Pesquisa e Reabilitação de Lesões Lábio Palatais/USP (HPRLLP/Centrinho, Bauru, SP) with the diagnostic hypothesis of VCFS. These individuals were selected based on the main characteristics of the VCFS, *i.e.*, typical facies, velopharyngeal insufficiency, behavioral disorders and/or learning disabilities. All of them were sporadic cases.

The 16 individuals with VCFS were submitted to a study protocol including standard semiology and family history, clinical, phonoaudiological and radiological exams. Learning disabilities, behavioral disturbances and mental retardation were assessed by psychological tests. Cardiac evaluation, including electrocardiogram and echodopplercardiogram, was carried out on all 16 individuals examined and those with cardiac alterations excluded from our study. The mean age of the group ranged from 6 to 32 years with a mean of 14 ± 5.9 years. Table 1 summarizes the clinical findings in our group.

We obtained GTG-banded chromosome preparations from lymphocyte cultures (Yunis, 1976) and at least 25 metaphases/prometaphases were analyzed per individual at the resolution level of 550 to 800 bands. FISH was performed using the 90 kb N25 (D22S75) cosmid probe that encompasses the DiGeorge/VCFS minimal critical region (Oncor, Inc., Gaithersburg, MD). Control probe pH 17 (D22S39) (Oncor, Inc., Gaithersburg, MD) was co-hybridized. For immunodetection, anti-digoxigenin fluorescent isothiocyanate-conjugated antibody was used. Chromosomes were counterstained with propidium iodide. Analyses were performed by two independent observers using a Carl Zeiss Axioskop Microscope equipped with a standard

Table 1 - Clinical findings present in individuals with velocardiofacial syndrome (VCFS) but without cardiac anomalies.

		Patients															
Clinical finding	(%)	01	02*	03	04	05	06*	07	08	09	10	11	12	13*	14	15	16
Age		32	16	13	12	11	12	10	15	22	08	14	17	15	06	15	13
Velopharyngeal insufficiency	100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Behavioral disturbances	100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Learning disabilities	62	-	+	+	-	+	+	+	+	-	-	+	-	+	-	+	+
Mental retardation	37	-	+	+	-	-	+	+	+	-	-	-	-	-	+	-	-
Delayed speech	32	-	-	-	-	+	+	-	+	-	-	+	-	-	+	-	-
Hearing loss	32	-	+	+	+	-	+	-	-	-	-	-	-	-	-	-	+
Small stature	6	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Pierre Robin Sequence	6	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Hypotonia	6	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Typical facies	100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Long face	100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hypertelorism	82	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	-
Narrow palpebral fissures	100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prominent nose	100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small mouth	56	+	+	+	+	+	+	+	-	-	-	-	-	-	+	-	+
Malar flatness	82	+	+	+	+	+	+	+	+	-	-	+	+	+	-	+	+
Vertical maxillary excess	32	-	-	+	+	-	-	+	+	-	+	-	-	-	-	-	-
Retrognathia	50	-	-	-	+	+	+	+	-	-	+	-	-	+	+	+	-
Microcephaly	19	-	-	-	-	-	-	-	-	+	+	-	+	-	-	-	-
Abundant scalp hair	62	-	+	+	+	+	-	+	+	-	-	+	+	-	+	+	-
Auricular anomalies	75	+	+	+	+	+	+	+	+	+	-	+	-	-	+	-	+
Cleft palate	62	+	-	+	+	+	+	-	-	-	+	-	+	-	+	+	+
Vertebral anomalies	62	-	+	+	+	+	-	-	+	+	+	+	-	-	-	+	+
Hyperextensibility of digits	25	+	-	-	-	-	-	+	-	-	-	+	-	-	+	-	-
Umbilical hernia	32	-	-	-	-	-	+	-	+	-	-	+	-	-	+	-	+
Inguinal hernia	13	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+
22q11.2 deletion (FISH)	62	+	+	+	-	+	+	+	-	-	+	+	-	+	+	-	-

(+) present; (-) absent; * 22q11.2 deletion detected with high-resolution banding.

fluorescence isothiocyanate (FITC) filter. Photographs were taken with Kodak Ektachrome 400 film.

A 22q11.2 deletion was identified in three (19%) individuals (02, 06 and 13) after GTG-banding. No other chromosomal abnormalities were detected. When FISH was used, ten (62%) individuals (01, 02, 03, 05, 06, 07, 10, 11, 13 and 14) showed the deletion (Table 1).

The critical VCFS region (DGCR) encompasses about 3 Mb, which is deleted in about 90% of individuals with VCFS (Morrow *et al*., 1995). In addition to the 3 Mb deletion, another 8% of cases have been found to present a smaller deletion of about 1.5 Mb (Carlson *et al.*, 1997a), and it has also been shown that in familial cases these smaller deletions were predominant (Adeyinka *et al*., 2004). The probe we used covered ~90 kb of the critical region and detected the majority of deletions. Nevertheless, the presence of deletions, which did not involve this segment, cannot be ruled out in our non-deletion individuals.

All our patients presented the characteristic clinical features of VCFS except for cardiac anomalies and, apart from this, there were no significant clinical difference between individuals with or without the 22q11.2 deletion, agreeing with previously reported data (Lindsay *et al.*, 1995a, Digilio *et al* 2003).

Frequencies of individuals without cardiac anomalies ranging from 25 to 70% have previously been reported among carriers of 22q11.2 deletions (Ryan *et al* 1997; McDonald-McGinn, 1999; Kitsiou-Tzeli *et al*., 2004; Bassett *et al*., 2005). To our knowledge this is the first study that determined the frequency of deletions in individuals with VCFS who had been selected on the basis of the absence of cardiac defects, and revealed the relatively high frequency of 62% deletions.

Testing for the presence of the 22q11.2 deletion is recommended for patients with two or more relevant clinical features of VCFS in any combination (typical facies, cardiac anomalies, palatal anomalies and learning disabilities), particularly when the typical facies is associated with palatal anomalies (Scambler, 2000). The high frequency of 22q11.2 deletion found in our small series of 16 individuals with VCFS without cardiac defects indicate that testing for deletions is recommended in such individuals.

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References

Adeyinka A, Stockero KJ, Flynn HC, Lorentz CP, Ketterling RP and Jalal SM (2004) Familial 22q11.2 deletions in DiGeorge/velocardiofacial syndrome are predominantly smaller than the commonly observed 3 Mb. Genet Med 6:517-520.

Bassett AS, Chow EWC, Husted J, Weksberg R, Caluseriu O, Webb GD and Gatzoulis M (2005) Clinical features of 78 adults with 22q11 deletion syndrome. Am J Med Genet 138:307-313.

Carlson C, Papolos D, Pandita RK, Faeda GL, Veit S, Goldberg R, Shprintzen RJ, Kucherlapati R and Morrow BE (1997a) Molecular analysis of velo-cardio-facial syndrome patients with psychiatric disorders. Am J Hum Genet 60:851-859.

Carlson C, Sirotikin H, Pandita R, Goldberg R, Mckie J, Wadey R, Pantajali SR, Weissman SM, Anyane-Yeboa K, Warburton D, Scambler P, Shprintzen RJ, Kucherlapati R and Morrow BE (1997b) Molecular definition of 22q11 deletions in 151 velo-cardio-facial syndrome patients. Am J Hum Genet 61:620-629.

Devriendt K, Fryns JP, Mortier G, Van Thienen MN and Keymolen K (1998) The annual incidence of DiGeorge/velocardiofacial syndrome. J Med Genet 35:789-790.

Digilio MC, Angioni A, De Santis M, Lombardo A, Gianotti A, Dallapiccola B and Marino B (2003) Spectrum of clinical variability in familial deletion 22q11.2 from full manifestation to extremely mild clinical anomalies. Clin Genet 63:308-313.

Driscoll DA, Spinner NB, Budarf ML, McDonald-McGinn DM, Zackai EH, Goldberg RB, Shprintzen RJ, Saal HM, Zonana J, Jones MC, Mascarello JT and Emanuel BS (1992) Deletions and microdeletions of 22q11.2 in velo-cardio-facial syndrome. Am J Med Genet 44:261-268.

Edelman L, Pandita RK and Morrow BE (1999) Low-copy repeats mediate the common 3 Mb deletion in patients with velocardiofacial syndrome. Am J Hum Genet 60:1076-1086.

Kitsiou-Tzeli S, Kolialexi A, Fryssira H, Galla-Voumvouraki A, Salavoura K, Kanariou M, Tsangaris GT, Kanavakis E and Mavrou A (2004) Detection of 22q11.2 deletion among 139 patients with Di George/velocardiofacial syndrome features. In Vivo 18:603-608.

Lindsay EA, Greenberg F, Shaffer LG, Shapira SK, Scambler PJ and Baldini A (1995a) Submicroscopic deletions at 22q11.2: Variability of the clinical picture and delineation of a commonly deleted region. Am J Med Genet 56:191-197.

Lindsay E, Goldberg R, Jurecic V, Morrow B, Carlson C, Kucherlapati RS, Shprintzen RJ and Baldini A (1995b) Velo-cardio-facial syndrome: Frequency and extent of 22q11 deletions. Am J Med Genet 57:514-522.

Lipson HA, Yuille D, Angel M, Thompson PG, Vandervood JG and Beckenham EJ (1991) Velocardiofacial (Shprintzen) syndrome: An important syndrome for the dysmorphologist to recognize. J Med Genet 28:596-604.

McDonald-McGinn DM, Kirschner R, Goldmuntz E, Eicher P, Gerdes M, Moss E, Solot C, Wang P, Jacobs I, Handler S, Knightly C, Heher K, Wilson M, Ming JE, Grace K, Driscole D, Pasquariello P, Randall P, Larossa D, Emanuel BS and Zackai EH (1999) The Philadelphia story: The 22q11.2 deletion: Report on 250 patients. Genet Couns 10:11-24.

Morrow B, Goldberg R, Carlson C, Gupta RD, Sirotkin H, Collins J, Dunham I, O'Donnel H, Scambler P, Shprintzen R and Kucherlapati R (1995) Molecular definition of the 22q11 de-

letions in velo-cardio-facial syndrome. Am J Hum Genet 56:1391-1403.

Ryan AK, Goodship JA, Wilson DI, Philip N, Levy A, Seidel H, Schuffenhauer S, Oechsler H, Belohradsky B, Prieur M, Aurias A, Raymond FL, Clayton-Smith J, Hatchwell E, McKeown C, Beemer FA, Dallapiccola B, Novelli G, Hurst JA, Ignatius J, Green AJ, Winter RM, Brueton L, Brondum-Nielsen K and Scambler PJ (1997) Spectrum of clinical features associated with interstitial chromosome 22q11 deletions: A European collaborative study. J Med Genet 34:798-804.

Sandrin-Garcia P, Macedo C, Martelli LR, Ramos ES, Guion-Almeida ML, Richeri-Costa A and Passos GAS (2002) Recurrent 22q11.2 deletion in a sibship suggestive of parental germline mosaicism in velocardiofacial syndrome. Clin Genet 61:380-383.

Scambler PJ (2000) The 22q11 deletion syndromes. Hum mol Genet 9:2421-2426.

Shprintzen RJ (1990) Velo-cardio-facial syndrome. In: Buyse ML (ed) Birth Defects Encyclopedia. Center of Birth Defects Information Services, Dover, pp 1744-1745.

Shprintzen RJ, Goldberg R, Young D and Wolford L (1981) The velocardiofacial syndrome: A clinical and genetics analysis. Pediatr 67:167-172.

Yunis J (1976) High resolution of human chromosomes. Science 191:1268-1270.

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