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Detection of 22q11.2 Deletion Among 139 Patients with Di George/Velocardiofacial Syndrome Features

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Abstract. *Cytogenetic and FISH analysis was performed in 139 patients to detect the pathognomonic of Di George/ Velocardiofacial syndrome (DGS/VFCS) deletion 22q11.2. An abnormal karyotype was revealed in 2/139 cases (47, XXY and 46, XX, 2p+). A deletion was found in 17/139 (12.2%) patients (14 males/ 3 females), inherited in 3 (2 maternal and 1 paternal). Patients with 22q11.2 deletion exhibited facial dysmorphic features (82%), congenital heart defects (70%), immunological problems (47%), multiple congenital anomalies (64%), hypocalcemia (47%), mental retardation/learning difficulties (35%), cleft palate/ velopharyngeal insufficiency (23.5%), seizures/hypotonia (23%) and growth retardation (12%). Among 56/139 patients with detailed available clinical data, the 22q11.2 deletion was confirmed in all cases with hypocalcemia and in over half of the cases with multiple congenital anomalies, immunological problems and hypotonia/seizures (70%, 60% and 57%, respectively). Genetic reevaluation of 39 patients without the 22q11.2 deletion contributed to the classification of 14 (37%) under different syndromes, emphasizing the need for stricter referral criteria.*

Di George and Velocardiofacial syndromes (DGS/VCFS) are the result of deletions or other rearrangements of chromosome 22q11.2 in over 90% of cases (1,2). The cardinal presenting symptoms and clinical features are cellular immunodeficiency due to thymus hypo-or aplasia, hypocalcemia because of parathyroid absence, congenital heart defect with high morbidity and mortality and typical faces (3,4). Increased frequency of psychiatric diseases, such as schizophrenia or bipolar disorders, have been observed in adult patients. The prevalence of DGS/VSFS is 1:4,000, but this figure may be underestimated because of the high observed rate of perinatal deaths in many cases with a severe congenital heart defect (6,7).

The spectum of clinical findings is very wide and more than 80 different congenital anomalies in various combinations have been reported in the literature, ranging from early neonatal death to very mild cases with only hypoparathyroidism or nasal speech (2,8-12). DGS/VCFS phenotypes represent the expression of developmental disturbances of the neural crest during the embryogenesis of the third and fourth pharyngeal pouch (13,14) and are attributed to the happloinsufficiency of one or more of the genes located at the chromosomal region 22q11.2. The estimated number of genes are over 100 and the DCS/VCFS are considered to be contiguous genes syndromes (15-18). The deletion is inherited in 5-10% of cases, but the observation of phenotypic differences among members of the same family carrying an identical deletion 22q11.2 is indicative of a more complicated molecular genetic basis of DGS/VCFS (19). It has also been shown that the clinical severity is not related to the length of the deletion (20,21), while there are reports of patients with DGS/VCFS phenotypes without detection of del 22q11.2 or point mutations, suggesting a heterogeneous genetic entity of happloinsufficiency of more than one gene in this region (2).

The 3MB 22q11.2 deletion is thought to be the most frequent among the interstitial submicroscopic deletions and can be easily identified by *in situ* fluorescent hybridization (FISH). To evaluate the frequency and clinical findings of DGS/VCFS, we undertook a retrospective analysis of children referred for the detection of 22q11.2 deletion with FISH.

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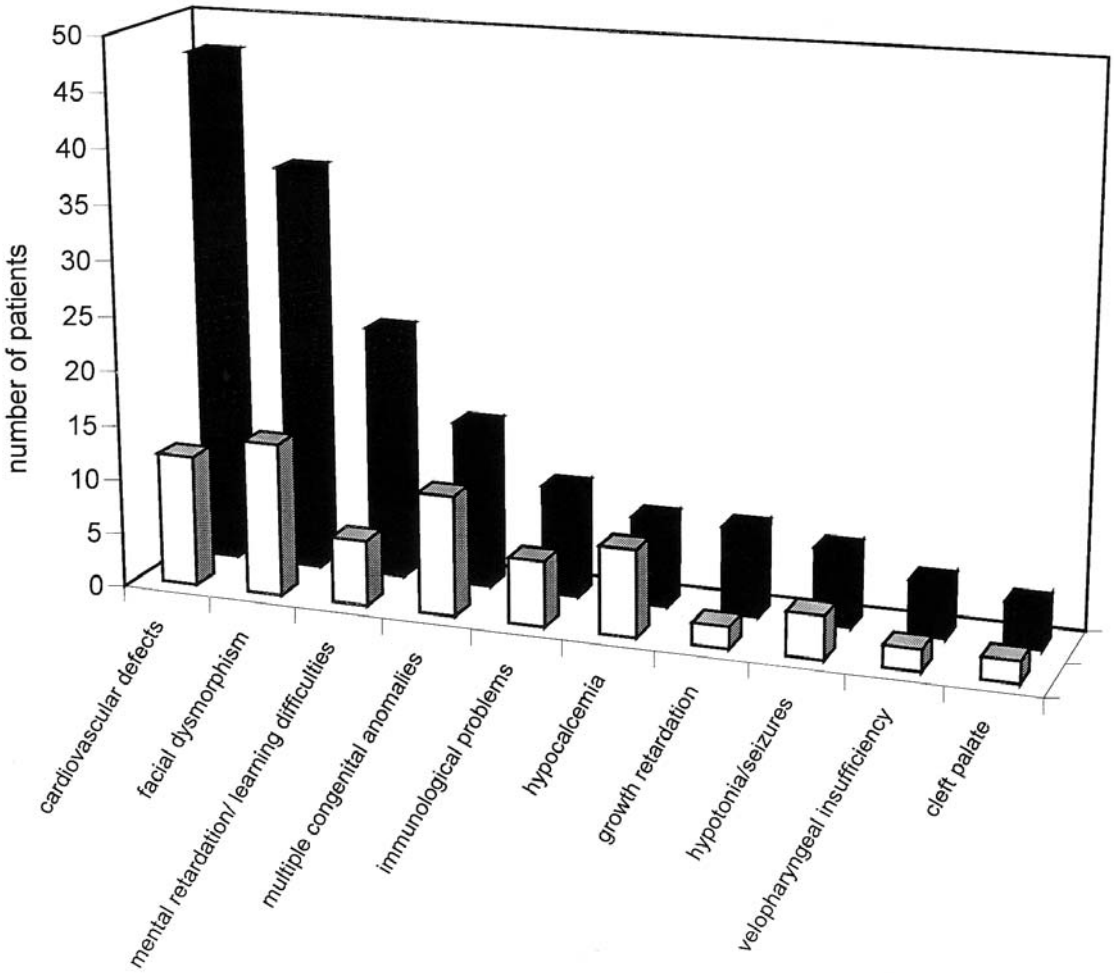

Figure 1. *Dysmorphic features of 56 patients with detailed available medical history (black bars) and 17 patients with del 22q11(white bars).*

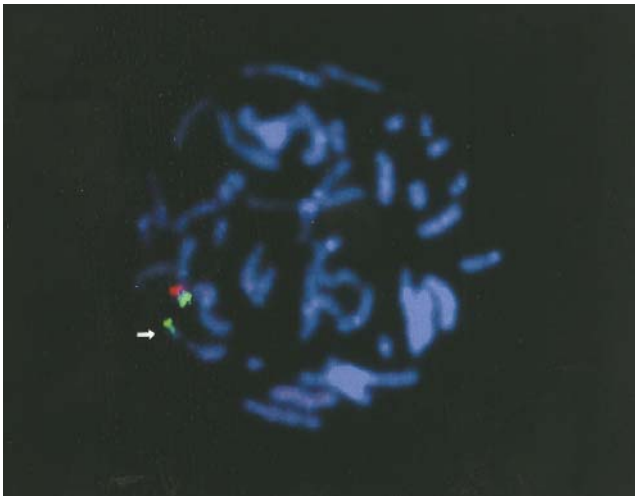

Figure 2. *FISH analysis in a patient with del 22q11.2. The normal chromosome 22, showing one red and one green (marker) hybridization signal and the deleted one showing only one green signal (marker).*

Patients and Methods

Clinical data were collected from 139 patients (82 males and 57 females), aged 10 days to 15 years, who were investigated to confirm the clinical diagnosis of DGS/VCFS. A detailed medical history was available only in 56/139 patients (40.3%), referred by paediatricians (32/56), cardiologists (20/56) and neurologists (4/56) for one or more of the following: congenital heart defects (47/56), facial dysmorphic features (37/56), mental retardation/learning difficulties (23/56), multiple congenital anomalies (15/56), hypocalcemia (10/56), immunological problems (10/56), growth retardation (8/56), hypotonia/seizures (7/56), velopharyngeal insufficiency (5/56) and cleft palate (4/56) (Figure 1).

Peripheral blood lymphocytes were cultured for karyotypic analysis and GTG banded chromosomes were studied. The detection of 22q11.2 deletion was revealed by FISH using the specific DNA probe TUPLE Di George (Oncor) according to the manufacturer's protocol. The same investigation was undertaken for the parents of the probands in whom a 22q11.2 deletion was detected.

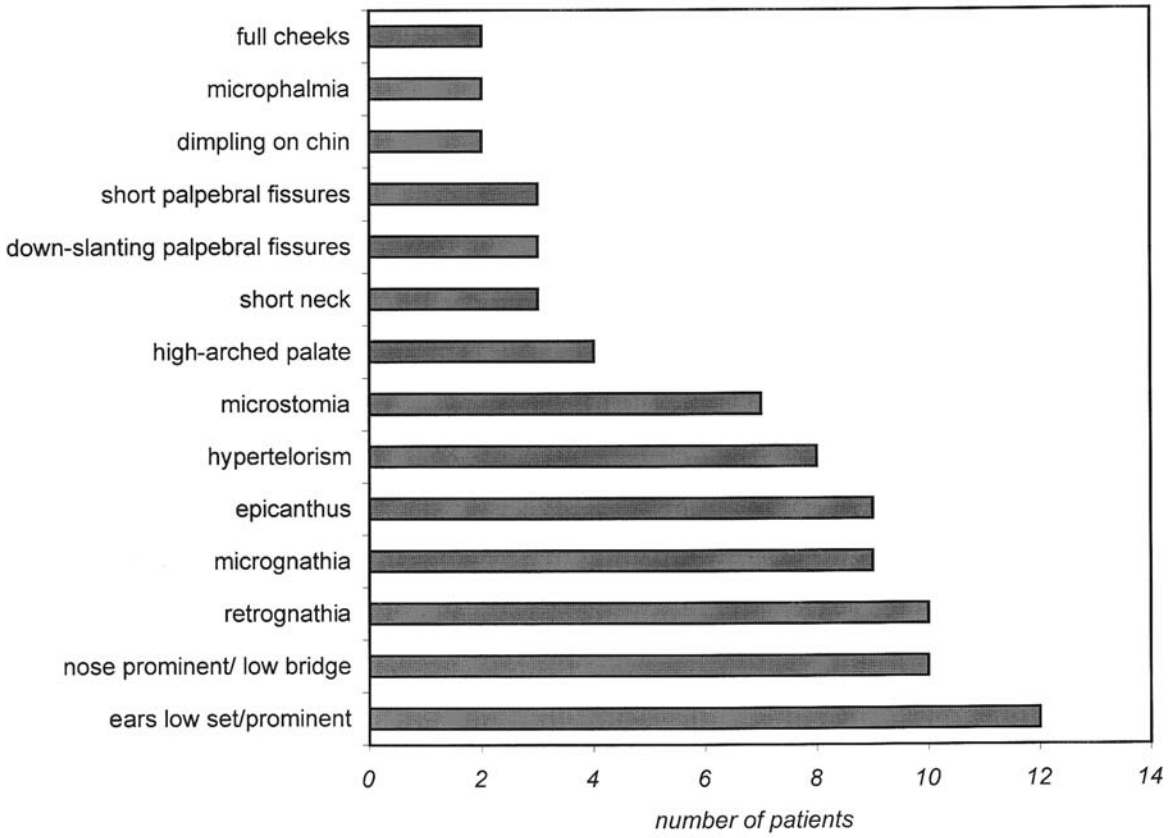

Figure 3. *Dysmorphic or peculiar facial features in 14/17 patients with del 22q11.2.*

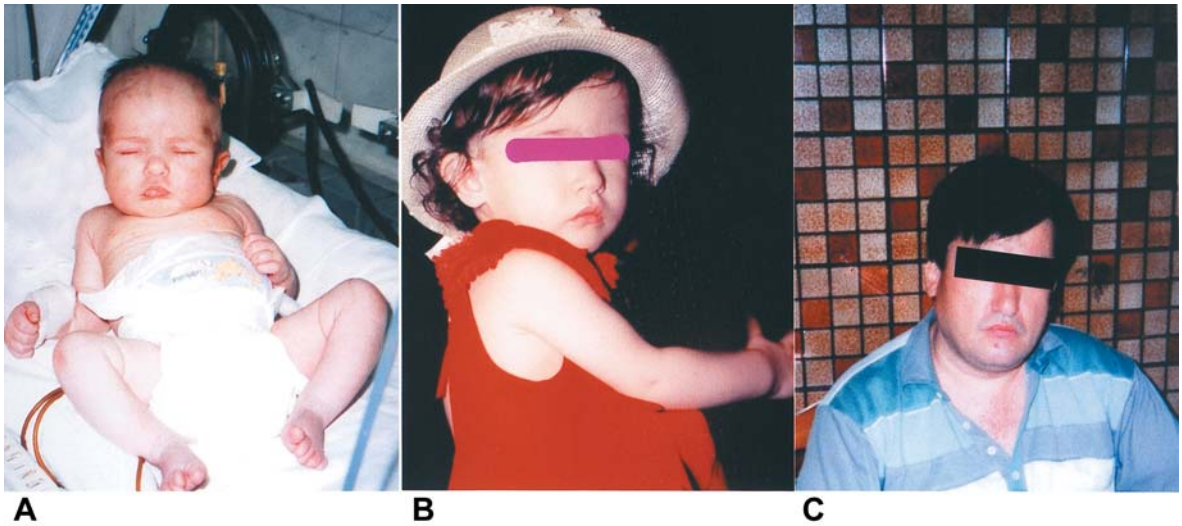

Figure 4. *A. A 1-month-old male with del 22q11.2, de novo. B. A 19-month-old girl with del 22q11.2, familial. C. Father of patient B.*

Results

Cytogenetic and FISH study. The cytogenetic analysis revealed a normal karyotype in 137/139 patiens (98.56%). A 5-year-old boy, who was referred because of congenital heart defect and mental retardation, had a 47,XXY karyotype. In a 14-month-old girl, with hypotonia and developmental delay, a *de novo* 46,XX,2p+ karyotype was found. FISH analysis using chromosome #2 painting showed that the additional material was derived from #2. A 22q11.2 deletion

Table I. *Spectrum of congenital anomalies in 17 patients with DGS/VCFS*.*

System	Anomaly	No. of patients
cardiovascular	ventricular septal defect	6
	Fallot's tetralogy	3
	pulmonary stenosis	2
	complex cardiopathy	2
	right aortic arch	1
	pulmonary hypoplasia	1
	patent ductus arteriosus	1
urogenital	pyelo-uretere-ectasis	2
	unilateral renal agenesis	1
	cryptorchidism	1
	hypospadias	1
	phimosis	1
limbs	long fingers	5
	overlapping fingers	2
	syndactyly, abnormal nails	2
brain	glioma	1
	hypoependymatic cyst	1
	ventricular dilation	1
velopharyngeal	larygomalacia	3
	cleft palate	2
	velopharyngeal insufficiency	2

*11/17 patients had multiple congenital anomalies

Table II. *Immunological investigation of patients with DGS/VCFS in the first month and 1 year of life.*

Patients	1st month*		1 year later **	
AK (♀)	CD3= 36%	IgG=503	CD3=36%	IgG=882
	CD4= 27%	IgA=10	CD4=23%	IgA=16
	CD8=12%	IgM=76	CD8=12%	IgM=46
	CD19=44%		CD19=40%	
	CD16=17%		CD16=24%	
MA(♂)	CD3=27%	IgG=837	CD3=77%	IgG=343
	CD4=20%	IgA<7	CD4=43%	IgA=41
	CD8=15%	IgM=23	CD8=28%	IgM=30
	CD19=34%		CD19=15%	
	CD16=17%		CD16=10%	
XA (♂)	CD3=32%	IgG=705	CD3=55%	IgG=952
	CD4= 23%	IgA=33	CD4=35%	IgA=70
	CD8= 8%	IgM=59	CD8=19%	IgM=68
	CD19=57%		CD19=31%	
	CD16=6%		CD16=18%	
KA (♂)	CD3=55%	IgG=389	CD3=55%	IgG=647
	CD4=35%	IgA=19	CD4=40%	IgA=23
	CD8=16%	IgM=93	CD8=15%	IgM=86
	CD19=32%		CD19=25%	
	CD16=12%		CD16=9%	
NA (♂)	CD3=34%	IgG=1135	CD3=46%	IgG=1057
	CD4=18%	IgA=47	CD4= 23%	IgA=28
	CD8=16%	IgM=67	CD8= 18%	IgM=180
	CD19=50%		CD19=39%	
	CD16=11%		CD16=17%	
MA (♂)	CD3=23%	IgG=710	CD3=70%	IgG=757
	CD4=14%	IgA=29	CD4=54%	IgA=73
	CD8=9%	IgM=52	CD8=12%	IgM=41
	CD19=60%		CD19=26%	
	CD16=14%		CD16=4%	

*Normal range: CD3 (60-85%), CD4 (41-68%), CD8 (9-23%), CD19 (4-26%), CD16 (3-23%), IgG (224-1072mg/dl), IgA (6-81mg/dl), IgM (31-135mg/dl).

**Normal range: CD3 (54-76%), CD4 (31-54%), CD8 (12-28%), CD19 (15-39%), CD16 (3-17%), IgG (575-1446mg/dl), IgA (32-245mg/dl), IgM (69-251mg/dl).

was detected is 17/139 patients (12.2%) (Figure 2). Investigation of all families showed that the deletion was inherited in three cases, two maternal and one paternal, while in the remaining families the deletion was *de novo*.

Presenting symptoms and clinical features of patients with 22q11.2 deletion. Among the patients with 22q11.2 deletion, 14 were males and 3 females, aged 10 days to 5 years (n=11), 6-10 years (n=2) and 11-15 years (n=4). The mean parental age at birth was 35 (father) and 26.5 (mother). The mean birth weight was 2684 g at 38 weeks of gestation (3.010 g males/2.480g females), while 4 were born before the 37th week (23.5%). During the perinatal/neonatal period, major complications of congenital heart defects were recorded in 11 cases (65%), hypocalcemia in 8 (46%), seizures and/or hypotonia in 4 cases (23%) and feeding problems associated with velopharyngeal anomalies in 4 cases (23%).

Growth retardation (body length and weight both <3 centile) was recorded in 2 children (2 and 3 years old) who had severe congenital heart disease (Fallot tetralogy and complex cardiopathy, respectively). Dysmorphic or peculiar facial features were observed in 14/17 patients (82%) (Figures 3,4). Information on the psychomotor development was available in 11/17 patients with deletion 22q11.2, since the remaining 6 were under 1 year of age and therefore no speculation was possible. Six out of 17 patients had developmental delay and/or learning difficulties (35%) but no severe behavioural problems were recorded.

A comparison of the presenting symptoms and clinical findings in patients with confirmed DGS/VCFS and the 56 patients with a detailed available medical chart is shown in Figure 1. The various congenital defects in DGS/VCFS are presented in Table I. Twelve out of 17 children with confirmed diagnosis of DGS/VCFS (70%) had one or more congenital heart diseases, most frequently ventricular septal defect (35%) and Fallot tetralogy (17.5%). In 11 children

Table III. *Frequency of main phenotypic abnormalities in DGS/VCFS.*

Phenotypic abnormalities	Goldberg *et al.* (1993) (n=9)	Leana-Cox *et al.* (1996) (n=12)	Ryan *et al.* (1997) (n=285)	Present study (n=17)
Facial dysmorphy		93%		82%
Cardiovascular	77%	68%	75%	70%
Thymus aplasia/ immunodeficiency		64%		47%
Parathyroid defects/ hypocalcemia	12%/ 20%	63%	60%	47%
Cleft/velopharyngeal insufficiency	100%	48%	9%/ 32%	23%
Urogenital			36%	35%

(64%), more that one congenital anomaly was observed in various combinations: skeletal (53%, most frequently of long fingers), urogenital (35%, with unilateral renal agenesis in 2/6), velopharyngeal (41%, cleft palate and/or velopharyngeal insufficiency) and CNS (17.5%). Disturbances of the lymphocytes' subpopulation were noted in 6 children aged 10 days to 1 year old, with mild clinical picture. They had a low percentage of T–lymphocytes (CD3+) and increased percentage of B-lymphocytes, but a normal count of natural killers and immunoglobulins. Only 2/6 children showed reduced lymphocyte proliferation. Their immunological profile was restored to normal by the first year of age and only one child, 2.5 years old with CD4+ < 400 mm^2, needed prolonged hospitalization because of serious infections (Table II).

Discussion

The retrospective analysis of 139 patients, referred for cytogenetic and FISH investigation by physicians for confirmation of the clinical diagnosis of DGS/VCFS, revealed that data for over half (83/139) were incomplete and limited to demographic information and the main reason for referral. This is possibly due to the fact that the majority of samples were sent to the laboratory by mail, prior to the completion of the necessary routine clinical and laboratory investigations. Regarding other more specific studies, such as kidney ultrasound and acouometry, we can assume that they had not been scheduled, waiting for confirmation of the DGS/VCFS diagnosis. Lack of useful information, such as psychomotor development, presents one of the usual disadvantages of most retrospective studies. It should also be noted that, of the 56 patients with detailed data available, the majority had not been examined by a clinical geneticist. Genetic revaluation of 39 patients without a 22q11.2 deletion contributed to the classification of 14 (37%) under the following genetic syndromes: Oculo-dental-digital, Alagille, Smith-Lemli-Opitz, Sotos, Costello, Langer-Giedion, Noonan, Acro-cranio-facial, Van Maldergem, OHDO and CHARGE.

A 22q11.2 deletion was detected in 17/139 patients referred for investigation (12.2%). It is interesting to note that, among the 56 patients with detailed available data, the comfirmation of clinical diagnosis differed when they were categorized according to the presenting symptoms and clinical findings: DGS/VCFS syndrome was confirmed in 8/8 patients with hypocalcemia (100%), in 6/10 with immunological problems (60%), in 4/7 with hypotonia/seizures (57%), in 2/4 with cleft palate (50%), in 11/15 with multiple congenital anomalies (70%), in 14/37 with dysmorphic facial features (38%), in 2/5 with velopharyngeal insufficiency (30%), in 6/23 with developmental delay/learning difficulties (26%), in 12/47 with congenital heart defects (25%) and in 2/8 with growth retardation (25%) (Figure 3).

In Table III our findings are compared with those of previous investigations (3,12,22). Concerning congenital heart defects, the limited number of our cases with 22q11.2 deletion does not permit the observation of less frequent cardiac anomalies, *i.e.* transposition of great vessels. The fact that only 30% of our cases with 22q11.2 deletion had no cardiac defect emphasizes the need for extended cardiological evaluation of any newborn who fulfils the diagnostic criteria of DGS/VCFS syndromes.

Also, the high frequency (23%) of urogenital anomalies detected in the present study, most of which require surgical management (Table I), suggests that ultrasound investigation of the kidneys should be considered in any newborn with a 22q11.2 deletion.

Palatal defects (cleft lip/velopharyngeal insufficiency) were observed in 23.5% of our patients, while higher frequencies have been reported in the literature (Table III). In a recent study, the del 22q11.2 was detected only in 1:99 children with cleft palate but without other features of VCFS (25). The authors, however, proposed that these children should be carefully followed up and evaluated by a geneticist in order to rule out VCFS.

Regarding immunological problems, only one patient required prolonged medical treatment with intravenous gamma-globulin administration up to the age of 2. It is interesting that one of our patients, with a 22q11.2 deletion but without neonatal immunological problems, developed lymphoma at 15 years and passed away 2 years later after rejection of a bone marrow transplantation. To our knowledge, this is the first case of DGS with a hematological malignancy.

In the present study, three families with inherited DGS/VCFS deletions were identified (17.6%). The comparison of the proband's phenotype to that of the carrier parent showed that the cardiac defect and developmental delay were more severe in the offspring. It has been suggested that a fertile parent with the 22q11.2 deletion represents the milder edge of the wide clinical spectrum of the syndrome. In 2/3 of the patients the deletion was maternal, a finding compatible with the literature (3). The frequency of inherited 22q11.2 deletions reported recently by Ryan *et al.* (3) is much higher than in previous studies (28% and 10%, respectively) (23, 24). It has been established that both parents should be investigated in any case of DGS/VCFS diagnosis, even if they do not have obvious phenotypic features or a positive family history of cardiac defects. Study of the siblings is indicated only in cases with inherited deletion.

In conclusion, genetic confirmation of DGS/VCFS with cytogentic studies and FISH analysis should be recommended to detect the parent/sibs with 22q11.2 deletion, who thereafter must be thoroughly examined by a cardiologist. In familial cases genetic counselling must be offered before the next pregnancy and prenatal diagnosis is indicated regardless of the severity of the parents or the previous child's phenotype.

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