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Screening of Patients at Risk for 22q11 Deletion

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A B S T R A C T

The aim of this study was to determine whether deletion 22q11.2 studies should become a part of a standardized diagnostic workup for selected groups of at risk patients. We prospectively investigated four cohorts of unselected patients referred because of: 1) congenital heart defect (CHD), 2) palatal anomalies, 3) hypocalcaemia, 4) dysmorphic features suggestive of del 22q11.2. Fluorescence in situ hybridization analysis revealed deletion 22q11.2 in 9.4% (6/64) patients with CHD. From 18 patients referred because of the hypocalcaemia, six (33.3%) had 22q11.2 deletion. In the group of 31 children with dysmorphic traits, the diagnosis was confirmed in two (6.4%) patients. None of the 58 children with palatal anomalies showed evidence of 22q11.2 deletion. Conclusions: Testing for the 22q11.2 microdeletion can be recommended in all patients with conotruncal heart defects and in patients with hypocalcaemia. It should be also considered in patients presenting only with dysmorphic traits suggestive of del 22q11.2, while screening in patients with cleft palate is not warranted.

***Key words**: chromosome 22q11.2 deletion, heart malformation, cleft lip, cleft palate, hypocalcaemia*

Introduction

Deletion 22q11.2 is the most common microdeletion syndrome, usually presenting with combination of features including cardiac defect, palatal abnormalities, craniofacial dysmorphism and developmental delay. Due to the variability of clinical expression, individuals with mild clinical presentation can pass unobserved[1]. Early diagnosis is important for appropriate medical attention including monitoring for complications and possibly for predicting surgical outcome[2,3]. It also gives the opportunity for timely genetic counselling and further testing including prenatal diagnosis in familial cases. A possible strategy for early identification of affected individuals is routine screening for 22q11.2 deletion in all infants presenting with one of the major clinical manifestations of the disorder. The purpose of this study was to evaluate the effectiveness of this approach by testing for del 22q11.2 unselected groups of patients with: heart defect, cleft palate, hypocalcaemia and dysmorphic traits suggestive of the del 22q11.2 phenotype (usually associated with developmental or behavioural problems and occasionally minor anomalies). To the best of our knowledge, this is the first study dealing with the screening for deletion 22q11.2 that covers all major clinical manifestations of the syndrome.

Patients and Methods

We screened 171 patients within a time interval of four years (from January 2001 to December 2004). There were 64 patients with congenital heart defects (CHD), 58 patients with palatal anomalies, 18 patients with hypocalcaemia and 31 patients with dysmorphic traits suggestive of del 22q11.2. All patients were evaluated by a clinical geneticist (I.B.). Based on her findings, the first three groups were divided into dysmorphic (at least three dysmorphic features from the del 22q11.2 phenotype) and non-dysmorphic subgroups. We also divided the patients with congenital heart defects according to the type of malformation into two groups for further analysis. Group I included 49 children with non-conotruncal heart defects, and group II 15 patients with conotruncal heart defects (CTD).

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Blood samples were taken after informed parental consent was obtained. In each patient we performed high-resolution chromosome analysis from phytohaemagglutinin stimulated lymphocyte cultures by standard methods. Fluorescence in situ hybridization (FISH) was performed with commercially available DNA probe mixture the LSI DiGeorge/VCFS region two-colour probe (Vysis, Downers Growe, IL, USA) according manufacturer's recommendations.

Results

A total of 171 patients were studied. There were 79 boys and 92 girls. The mean age was 5 years and 5 months (range from 4 days to 17 years and 10 months). Clinical evaluation classified 112 patients as dysmorphic, while 59 patients were classified as non-dysmorphic.

FISH analysis revealed 22q11.2 deletion in 14 cases out of 171 investigated patients (8.2%). There were 9 females and 5 males, aged from 17 days to 11 years and 2 months. All identified patients with 22q11.2 deletion belonged to the dysmorphic subgroup (Table 1). The frequency of the deletion among dysmorphic patients was 12.5% (14/112).

In the group of patients with congenital heart defects, deletion 22q11.2 was found in 9.4% of patients (6/64). The prevalence of deletion in the dysmorphic CHD patients was 15.4% (6/39). Table 2 provides a list of the

TABLE 1
CLINICAL FINDINGS IN PATIENTS WITH 22q11.2 DELETION

	Patients in CHD group						Patients in hypocalcaemia group						Patients in dysmorphic group		Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	14
Cardiac defect	+	+	+	+	+	+	–	–	–	–	–	–	–	–	6
Partial immunodeficiency	–	–	–	–	+	–	–	+	–	+	+	–	–	+	5
Hypocalcaemia	–	–	–	–	–	–	+	+	+	+	+	+	–	–	6
CP/CLP	–	–	+	–	–	–	–	–	–	–	–	–	–	–	1
Developmental delay, learning difficulties, behavioural problems	–	+	–	–	–	+	–	–	–	+	+	+	+	+	7
Minor anomalies	–	+	+	–	+	–	–	+	–	–	+	–	+	–	6
Dysmorphic features	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Growth retardation	–	–	–	+	–	–	+	+	–	–	–	+	+	–	5
Mental retardation	–	+	–	–	–	–	–	–	–	+	+	+	–	–	4
Other	–	+	–	–	–	–	+	–	–	+	–	–	–	–	3

CHD – congenital heart defect, CP – cleft palate, CLP – cleft lip and plate

TABLE 2
CHROMOSOME AND FISH STUDIES IN THE GROUP OF PATIENTS WITH NON-CONOTRUNCAL HEART DEFECTS

	Patients	Dysmorphic			Non-dysmorphic		
CHD		No	22q11.2	Other	No	22q11.2	Other
ASD	15	11	–	Opitz-Frias sy	4	–	–
PDA	5	3	–	–	2	–	–
CoA	4	2	–	45,X	2	–	–
PS/PA	4	3	1	–	1	–	–
AVSD	3	2	–	–	1	–	–
AS	3	2	–	–	1	–	–
ASD, PS, AS	1	1	–	7q11.2	–	–	–
ASD, VSD	2	–	–	–	2	–	–
VSD	12	5	1	–	7	–	–
Total	49	29	2	3	20	–	–

AS – aortic stenosis, ASD – atrial septal defect, AVSD – atrioventricular septal defect, CHD – congenital heart defect, CoA – coarctation of the aorta, PDA – patent ductus arteriosus, PS – pulmonary stenosis, VSD – ventricular septal defect

TABLE 3
CHROMOSOME AND FISH STUDIES IN THE GROUP OF PATIENTS WITH CONOTRUNCAL HEART DEFECTS

CTD	Patients	Dysmorphic			Non-dysmorphic		
		No	22q11.2	Other	No	22q11.2	Other
TGA	3	2	–	–	1	–	–
ToF	9	7	4	–	2	–	–
IAA	2	1	–	–	1	–	–
DAA	1	–	–	–	1	–	–
Total	15	10	4	–	5	–	–

CTD – conotruncal heart defect, DAA – double aortic arch, IAA – interrupted aortic arch, ToF – tetralogy of Fallot, TGA – transposition of the great arteries

non-conotruncal heart defects in the patients investigated and the results of chromosome and FISH studies. Microdeletion 22q11.2 was found in two patients, one with pulmonary stenosis (PS), and one with ventricular septal defect (VSD) (2/49 or 4.1%). In addition, we identified one patient with Turner syndrome and one patient with Williams syndrome. One patient with atrial septal defect (ASD) was clinically diagnosed as having Opitz Frias syndrome, but FISH analysis for the TUPLE1 locus in this patient did not reveal 22q11.2 deletion. Table 3 summarizes findings in 15 patients who had conotruncal heart defect. Among them the prevalence of 22q11.2 deletion was 26.6% (4/15). All four patients with microdeletion had tetralogy of Fallot (ToF).

None of the 58 patients with palatal anomalies (28 with cleft palate and 30 with cleft lip and plate) showed evidence of 22q11.2 deletion. In the dysmorphic subgroup of 27 patients, chromosome analysis of one female patient with cleft lip/palate and dysmorphic traits revealed r(18)(p11.2q23) karyotype.

In the group of patients with hypocalcaemia, deletion 22q11.2 was discovered in 6 of 18 patients (33.3%). Considering just the dysmorphic subgroup, microdeletion was present in 40% (6/15) of patients.

In the group of 31 children with dysmorphic traits from the del 22q11.2 phenotype spectrum, the deletion was discovered in two (6.4%) patients. One patient with short stature, and facial features suggestive of velocardiofacial syndrome (VCFS) including short palpebral fissures, ptosis, high arched palate, and velopharingeal insufficiency was diagnosed as being mosaic for Turner syndrome (45,X/46,XX).

Discussion

We have prospectively investigated four unselected cohorts of patients presenting with: 1) heart defect, 2) cleft palate, 3) hypocalcaemia and 4) dysmorphic features suggestive of del 22q11.2. The objective of the study was to determine should 22q11.2 deletion studies become a part of a standardized diagnostic workup for the subset of patients presenting with the major clinical manifestations of the del 22q11.2 spectrum.

A total of 14 patients were found to have a 22q11.2 deletion, all without visible cytogenetic abnormalities. There were six patients with congenital heart defects, six patients with hypocalcaemia and two patients with dysmorphic traits.

Congenital heart disease is a highly characteristic feature of del 22q11.2 syndrome, occurring in about 75–88% of all patients[4,5]. Several studies have reported prevalence of 22q11.2 microdeletion in unselected CHD patients[6–10]. Large variations in prevalence rates (from 1.5 to 43.5 %) are mainly due to the sample differences (selection of CHD studied, age of patients, presence of dysmorphic traits, and/or other extracardiac symptoms, etc). The high frequency of 43.5% found by Johnson et al.[9] is probably due to the study design, because he investigated a selected group of patients that were referred to a cytogenetic laboratory for evaluation. The prevalence rate of 1.5% found in the population-based study by Botto et al.[7] most likely reflects the true prevalence in the newborn population. In our patients with CHD, FISH analysis revealed 22q11.2 deletion in 9.4% (one patient with PS, one with VSD and four patients with ToF). In the dysmorphic group the prevalence was 15.4%. Our results are in accordance with the findings of a similarly designed study by Fokusten et al.[8] who found del 22q11.2 in 8% of patients with unselected CHD and 17.6% in the subset of patients with extracardiac symptoms.

About 14–18% of patients with del 22q11.2 have the perimembranous type of VSD[5,11,12]. We found one patient with deletion among 12 patients presenting with VSD (8.3%). In a cohort of 125 patients with VSD McEhinney et al.[13] found the deletion in 10% of cases.

It is now well established that conotruncal heart defects are the most typical cardiac malformations associated with the 22q11.2 deletion[4,5]. In our study 26.6% patients with CTD carried the deletion. The incidence of 22q11.2 deletion in patients with CTD in other studies shows a wide range from 0% up to 48%[14–19]. Here again the different rates are due to the study design. Debrus et al.[14] investigated only non-syndromic familial cases of CTD and found no deletion. High rates are found in studies that investigated only the newborn population or selected CTDs known to be associated more often with del 22q11.2[17,19,20].

Given the high frequency of del 22q11.2 in patients with CTD, some authors have suggested that all patients with CTD should undergo screening for the deletion[21,22]. Other authors advocate screening only in patients with additional extracardiac symptoms[6,8]. Four patients with del 22q11.2 and CTD in our sample were found among 10 patients in the dysmorphic subgroup. Still, most of the clinical features were quite mild and could pass unobserved if not specifically looked for by an experienced clinician. Exceptionally mild dysmorphic features were seen in a girl with pulmonary stenosis. She had hooded eyelids, proturberant ears, and slight micrognathia, but her facial appearance was otherwise normal. There were no hypocalcaemia, or clinical signs of impaired immunity, although subsequent laboratory investigation revealed mild partial depression of cellular immunity. Her somatic and intellectual development was normal. Recent study of adult CTD patients found that clinical evaluation inaccurately predict microdeletion in some FISH negative patients and incorrectly predicted absence of the deletion in some FISH positive patients. In half (3/6) of the detected patients with del 22q11.2 typical dysmorphic features were absent[23]. In view of the high frequency of del 22q11.2 among CTD patients, the fact that clinical diagnosis is unreliable, and the fact that patients go undiagnosed into adulthood, we think that screening for 22q11.2 should be considered in all CTD patients.

Palatal anomalies including velopharyngeal incompetence or submucosal cleft are often found in del 22q11.2, but overt cleft palate or cleft lip/palate is found only in 2–11% of patients[4,5,24]. In our group of 58 patients with oral clefts no deletion of 22q11.2 region was detected indicating that among these patients deletion rarely occurs. Other studies also suggest that deletion 22q11.2 is rarely found among patients with oral clefts[25,26]. Ruiter et al.[27] found one patient with del 22q11.2 out of 58 children with isolated cleft palate. A larger study comprising 101 patients with isolated cleft palate found only three patients (2.8%) with the deletion[28].

It is remarkable to note that of 18 unselected patients referred because of hypocalcaemia, six had 22q11.2 deletion. The age at diagnosis ranged from 2 months to 11 years and two months. The older age of diagnosis in four of our patients indicates that the risk of hypocalcaemic episodes is often present beyond the neonatal period. All patients were referred for evaluation because of convulsions. None of them had a heart defect or cleft palate. Two had sensorineural hearing loss, and three had partial immunodeficiency with no evident clinical manifestations. Of four older patients that could be formally tested, three showed mild to borderline mental retardation. One female patient had mild dysmorphic features, and no additional findings from the del 221q11.2 phenotype. Her intellectual development was normal. Hypoparathyroidism and facial dysmorphism have occasionally been described as the only sign of the del 22q11.2 syndrome[29,30]. High incidence of 22q11.2 deletion in patients with hypocalcaemia (neonatal, transient, latent, permanent or late-onset) was also noted by the some other authors[4,31,32]. Although based on a small sample of patients, our results indicate that individuals presenting with hypocalcaemia with no obvious reason, should be also tested for 22q11.2 deletion.

In the group of 31 patients with dysmorphic features of 22q11.2 microdeletion syndrome we discovered two patients with 22q11.2 deletion. The age at diagnosis was 3 years and four months and 4 years and three months respectively. Both patients had mild developmental delay. Although dysmorphic features were present in all patients discovered in our series, they were often mild and could easily be overlooked if not evaluated by an experienced clinical geneticist. Further studies of the group of patients presenting with dysmorphic features, minor unspecific anomalies and/or developmental delay by molecular methods would be of interest because some of them could have atypical deletions in the 22q11.2 region[33].

To summarise, although the number of investigated patients in our study was not large, we think that the described findings can well support our view that it is appropriate to evaluate for 22q11.2 deletion syndrome patients with apparently isolated conotruncal caridac defects and patients presenting with hypocalcaemia. As dysmorphic features could be the only presenting sing of del 22q11.2, we also advocate testing for del 22q11.2 in this group of patients. Del 22q11.2 is rarely detected in patients with cleft palate and screening of this group of patients is not warranted.

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PROBIR DEL 22q11 U RIZIČNIM SKUPINAMA BOLESNIKA

S A Ž E T A K

Cilj ovog rada je utvrditi da li analiza delecije 22q11.2 treba postati dio rutinskog dijagnostičkog ispitivanja kod selekcioniranih skupina rizičnih bolesnika. Prospektivno smo ispitali 4 skupine neselekcioniranih bolesnika upućenih zbog 1) kongenitalne srčane mane, 2) anomalije nepca, 3) hipokalcemije, 4) dizmorfičnih obilježja 22q11.2 delecije. Metodom fluorescentne in situ hibridizacije otkrivena je delecija 22q11.2 kod 9,4% (6/64) bolesnika sa srčanom manom. Od 18 bolesnika s hipokacemijom šest (33,3%) je imalo 22q11.2 deleciju. U skupini od 31 djeteta s dismorfijom dijagnoza je potvrđena kod dva (6,4%) bolesnika. Kod nijednog od 58 djece s anomalijama nepca nije utvrđena 22q11.2 delecija. Analizu mikrodelecije 22q11.2 preporuča se napraviti kod svih bolesnika s konotrunkalnom srčanom manom i bolesnika s hipokalcemijom. Također bi trebalo uzeti u obzir i bolesnike kod kojih su prisutna samo dismorfična obilježja delecije 22q11.2, dok probir kod bolesnika s rascjepom nije opravdan.