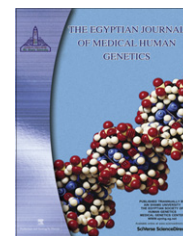




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ORIGINAL ARTICLE

Chromosome 22 microdeletion in children with syndromic congenital heart disease by fluorescent in situ hybridization (FISH)

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Hypocalcemia;
Velocardiofacial syndrome

Abstract Congenital heart diseases (CHDs) are the most common of all birth defects. Congenital heart disease may occur as an isolated malformation or may be part of a syndrome. One of the most common syndromes associated with CHDs is the 22q11.2 microdeletion syndrome, the various conditions associated with del22q11 include DiGeorge syndrome (DGS), velocardiofacial syndrome (VCFS), conotruncal anomaly face syndrome (CTAFS), and others. The abnormalities associated with this syndrome include parathyroid hypoplasia, thymic hypoplasia, immune defect, cleft palate, and abnormal facies. The cardiac defects are usually derived from conotruncus.

The aim of the study was to detect the prevalence and the most common or frequent clinical manifestations of chromosome 22q11.2 microdeletion among children with syndromic congenital heart disease.

The study was conducted on 20 children with syndromic CHD presenting to the Menoufiya University Hospitals, Egypt. Their ages ranged from 10 days to 12 years. Cytogenetic study and fluorescence in situ hybridization (FISH) were performed in the patients. The study revealed that 2 patients were with chromosomal aberrations [one with 46,XY, add (13)(p13) & the other with 47,XX, +13]. In addition, FISH revealed 4 patients (20%) with 22q11.2 microdeletion syndrome.

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The congenital heart malformations detected in patients with 22q11.2 microdeletion were somewhat unexpected and included VSD, ASD, PDA, and double outlet right ventricle. The most frequent extracardiac features were hypocalcemia, microcephaly, brain atrophy, epicanthus, low set posteriorly rotated ears, micrognathia, and anemia. The extracardiac features were in some cases subtle.

It is concluded that 22q11.2 microdeletion is not uncommon and its manifestations are highly variable. This entails that screening for the microdeletion by FISH should be performed in all patients with syndromic CHD especially those with hypocalcemia, microcephaly, brain atrophy, epicanthus, low set ears, posteriorly rotated ears, micrognathia, and anemia. In addition, patients with minor features and those with non-conotruncal heart disease should not be excluded from the screening for 22 microdeletion.

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1. Introduction

Congenital heart disease (CHD) affects about 1% of newborn children and is the leading cause of death in infants under 1 year of age [1]. In most patients, CHDs may be isolated but about 33% have associated anomalies (syndromic CHD). One of the most common syndromes associated with CHD is 22q11.2 microdeletion syndrome [2].

22q11DS is the most common microdeletion syndrome in man [3] with a minimum prevalence rate of one in 4000 live births [4].

The phenotype of 22q11DS encompasses DiGeorge syndrome (cardiac anomalies, hypoparathyroidism, immunodeficiency), velocardiofacial syndrome (VCFS) (pharyngeal dysfunction, cardiac anomaly, dysmorphic facies) [5], Conotruncal anomaly face syndromes [6], Cayler Cardiofacial syndrome (asymmetric crying facies) [7,8], CHARGE syndrome (coloboma, heart defects, atresia of the choanae, retardation of growth, genitourinary problems and ear abnormalities) [9], and autosomal dominant Opitz G/BBB syndrome (Hypertelorism-Hypospadias syndrome) [10].

Over 180 clinical features have been associated with VCFS but there is no single finding or group of findings that occur in all affected individuals. Cardinal features that raise diagnostic suspicion in newborns include cleft palate when seen with conotruncal heart defects, especially if accompanied by hypocalcemia [11].

Approximately 75% of patients with 22q11DS have CHD, typically involving the conotruncus and include lesions such as Tetralogy of Fallot and truncus arteriosus. Associated anomalies of the aortic arch, ductus arteriosus, and pulmonary arteries are more frequent in these patients [12].

Characteristic facial features include increased vertical length of the face, distinctive nasal configuration, hooded eyelids, hypertelorism, ear abnormalities, and retrognathia. The nasal root is prominent with a broad or "tubular" midportion, bulbous tip, and hypoplastic alae nasae. Asymmetric crying facies, craniosynostosis, and flat malar eminences are also seen [11].

Hypocalcemia, due to hypoparathyroidism and a variably severe immune deficiency due to thymic hypoplasia [13] are characteristic features of 22q11.2 DS.

Other clinical manifestations of 22q11.2 DS include abnormalities of the palate (such as cleft palate and velopharyngeal incompetence), eye, ear, nose, respiratory tract, genitourinary system, and the skeletal system. 22q11.2 DS can

also involve the nervous system with variable effects on behavior, psychosocial development and cognitive function, and speech.

22q11.2 microdeletion has also been implicated in isolated non-syndromic CHD [12]. However, caution should be taken before stating a causal relationship between del22 and isolated CHDs because the phenotypical expression of 22q11.2 DS is extremely variable and the analysis of large series from the literature shows that patients with CHD and del22 always present one or more additional phenotypical anomaly of the syndrome [14].

The aim of this study is to detect the prevalence and clinical manifestations of chromosome 22q11.2 microdeletion among children with syndromic congenital heart disease.

2. Patients and methods

2.1. Patients

The study was conducted on 20 children with syndromic congenital heart disease, i.e. congenital heart disease with at least one extracardiac anomaly, they were selected from the Menoufiya University Hospitals, Egypt, during the period from 2007 to 2009.

Exclusion criteria: Patients with CHD associated with known environmental risk factors, e.g. maternal diabetes were excluded from the study. Likewise, patients with well known syndromes, e.g. Down syndrome were excluded.

2.2. Methods

All studied patients (after having written informed consents from parents) were subjected to the following: A detailed history, thorough clinical examination and laboratory investigations including complete blood picture, serum calcium and genetic studies. Radiological investigations included chest X-ray and pelvic/abdominal ultrasonography. Specific investigations were ordered for each case as needed.

All patients were subjected to a high resolution cytogenetic study using G-banding and fluorescence in situ hybridization (FISH).

FISH technique was performed using a fluorophore labeled locus specific identifier (LSI) TUPLE1 (HIRA) probe/ARSA (Arylsulfatase A) dual color DNA probe which hybridizes to band 22q11.2 of chromosome 22 (LSI TUPLE Spectrum

Table 1 Demographic data of the studied group.

Variable	No.	%	Range	Median	Mean
Age	–	–	10 days–12 years	1 year	22.7 months
<i>Sex</i>					
Male	9	45	–	–	–
Female	11	55	–	–	–
<i>Gestational age</i>					
Preterm	1	5	–	–	–
Full term	19	95	–	–	–
Post term	0	0	–	–	–
<i>Birth weight for gestational age</i>					
SGA*	8	40	–	–	–
AGA**	12	60	–	–	–
LGA***	0	0	–	–	–

* Small for gestational age.

** Appropriate for gestational age.

*** Large for gestational age.

Orange) and to band 22q13 (ARSA Spectrum Green) of chromosome 22. The hybridized probe fluoresces with moderate to bright intensity both in interphase nuclei and on metaphase chromosomes. In interphase nuclei of normal cells, the probe generally appears as 2 distinct orange signals and a distinct green signal. The ARSA is specific to the 22q13 band that maps very close to the telomeric end of chromosome 22 and is directly labeled with spectrum green and it is used as an internal control and also to evaluate 22q13 as doses of green. The probe was hybridized to metaphase and interphase cells. The absence of the orange pink signal on chromosome 22 indicates deletion of the TUPLE1 locus at 22q11.2.

3. Results

The results of this study are illustrated in Tables 1–9 and Figs. 1–5.

The study included 20 patients. Their ages ranged from 10 days to 12 years with a mean of 22.7 months. Nine cases

Table 3 Anthropometric measurements of the studied group.

	Weight		Length		Head circumference	
	No.	%	No.	%	No.	%
<3rd percentile	12	60	12	60	13	65
>97th percentiles	0	0	0	0	1	5
Normal percentiles	8	40	8	40	6	30

(45%) were males and 11 (55%) were females. Only one case (5%) was preterm. Eight cases (40%) were small for gestational age (SGA) whereas 60% were appropriate for gestational age (AGA). No patient was large for gestational age (LGA) Table 1.

Family history (Table 2) revealed that consanguinity was positive in 4 cases (20%). Maternal age ranged from 20 to 38 years with a mean of 27.1 ± 4.24 years. Paternal age ranged from 25 to 45 years with a mean of 34.25 ± 5.35 years. Only 2 women (10%) were of old age (≥ 35 years) while the majority (90%) were of middle age (20–34 years). No mother had a young age (<20 years). There was no family history of congenital heart disease in our study group. History of other congenital anomalies was present in 20% of cases. These included myelomeningocele, an unknown lung anomaly, and polydactyly in 2 cases (10%). Family history of motor or mental handicap was present in 50% of cases. History of sib death was present in 2 cases (10%). Maternal history of abortion was present in 25% of cases. History of stillbirth was present in one case (5%). No history of preterm labor was reported.

Anthropometric measurements of patients showed that 60% of patients had weights below the 3rd percentiles. Sixty percent of children had short stature (all were proportionate). Sixty-five percent of children had a small head circumference (less than the 3rd percentiles). Thirty percent of children had normal head circumference and only one child (5%) had a large head circumference (Table 3).

As to the type of congenital heart disease in our study group, VSD, pulmonary stenosis, PDA, and ASD plus VSD

Table 2 Family history of the studied group.

	No.	%	Range	Mean	SD
<i>Consanguinity</i>					
+ve	4	20	–	–	–
–ve	16	80	–	–	–
<i>Parental age</i>					
Maternal age (y)	–	–	20–38	27.1	4.24
Paternal age (y)	–	–	25–45	34.25	5.35
<i>Maternal age groups</i>					
Young age (<20 years)	0	0	–	–	–
Middle age (20–34 years)	18	90	–	–	–
Old age (≥ 35 years)	2	10	–	–	–
<i>Medical history of the family</i>					
History of congenital heart disease	0	0	–	–	–
History of other congenital anomalies	4	20	–	–	–
History of motor or mental handicaps	10	50	–	–	–
History of sib death	2	10	–	–	–
<i>Obstetric history</i>					
Abortion	5	25	–	–	–
Preterm labor	0	0	–	–	–
Still birth	1	5	–	–	–

Table 4 Types and prevalence of CHD among patients.

Type of CHD	No.	%
VSD	2	10
Pulmonary stenosis (PS)	2	10
PDA	2	10
ASD + VSD	2	10
Fallot tetralogy	1	5
Subvalvular aortic stenosis	1	5
Double outlet right ventricle	1	5
PDA + 2 ASDs	1	5
VSD + Coarctation of aorta	1	5
ASD + PS + Coarctation of aorta	1	5
Fallot tetralogy + PDA + 2 ASDs	1	5
Tricuspid atresia + VSD + ASD + PS	1	5
Supravalvular aortic stenosis + ASD + branch pulmonary stenosis	1	5
ASD + inlet VSD + outlet VSD + pulmonary atresia + hypoplastic pulmonary arteries + right sided aortic arch + complete atrioventricular septal defect	1	5
Shone complex (Coarctation of aorta, subvalvular AS, parachute mitral valve) + thickened tricuspid valve leaflets with abnormal insertion of its septal leaflet	1	5
ASD + Aortic regurgitation + mitral regurgitation + non-compaction of the left ventricle	1	5
Total	20	100

Table 5 Results of karyotype analysis in the studied group.

Karyotype	No.	%
<i>Normal</i>		
Male (46,XY)	8	40
Female (46,XX)	10	50
<i>Abnormal</i>		
Numerical 47, XX, +13 (trisomy13)	1	5
Structural 46,XY, add (13)(p13)	1	5
Total	20	100

were the most common types; each existed in 2 cases (10%) (Table 4).

High resolution cytogenetic study revealed that 8 patients (40%) had a normal male karyotype (46, XY) while 10 patients (50%) had normal female karyotype (46, XX). The third patient had a numerical chromosomal aberration in the form of trisomy 13 (47, XX, +13). The fifth patient had a structural chromosomal aberration in the form of 46, XY, add (13)(p13) (Table 5 and Fig. 1A and B).

These two patients with chromosomal aberrations on conventional cytogenetic study were excluded from FISH study. FISH study revealed that 4 patients (20%) had chromosome 22 microdeletion while 14 patients (70%) had normal chromosome 22 (Table 6).

The ages of our patients with 22 microdeletion ranged from 2.5 months to 5 years. They were 3 females and 1 male. They were all full term with normal birth weight. Consanguinity was negative in all cases and there was no family history of CHD. However, family history of genetic disease or developmental delay was present in 3 cases. History of abortion was reported in only 1 patient's mother (Table 7).

The cardiac defects in patients with 22q11.2 microdeletion included PDA, VSD, and double outlet right ventricle, and ASD plus PDA (Table 8).

The most frequent extracardiac features in patients with 22q11.2 DS were hypocalcemia, microcephaly, brain atrophy,

Table 6 Prevalence of chromosome 22 microdeletion, by FISH technique, among all patients with syndromic CHD.

	No.	%
Chromosome 22 microdeletion by FISH	4	20
Normal chromosome 22 by FISH	14	70
Chromosome 22 not tested by FISH	2	10
Total	20	100

epicanthus, low set ears, micrognathia, posteriorly rotated ears, and anemia. These features occurred in 75% of cases. Less frequent features (occurred in 50% of cases) were delayed motor and mental milestones, upward slanting palpebral fissure, recurrent seizures, hypertelorism, attached ear lobule, anteverted nostrils, PDA, and lymphopenia. Most features occurred in only one patient (25%). These included decreased weight and decreased length, agenesis of the corpus callosum, absent septum pellucidum, asymmetric crying face, malar flattening, broad forehead, deep set eyes, triangular face, partial synophrys, hooding of the eyelids, antimongoloid slant, narrow palpebral fissure, arched eyebrows, broad nasal bridge, depressed nasal bridge, hypoplastic ala nasi, skin tag on the helix, short vertical diameter of ears, umbilical hernia, epigastric hernia, anal stenosis, microstomia, dental caries, delayed teeth eruption, laryngeal web, simian crease, camptodactyly of 5th finger, and tapering fingers (Table 9 and Fig. 4).

It is to be noted that all patients with 22q11.2 microdeletion had normal thymic shadow on X-ray.

4. Discussion

Over the past decade, there have been major breakthroughs in the understanding of inherited causes of CCVDs, including the identification of specific genetic abnormalities for some types of malformations [15].

Many patients with CHD are managed as just "cardiac cases", though their cardiac disease may be nothing but the

Table 7 Demographic and historical data of patients with 22q11.2 microdeletion.

Serial no.	Age	Sex	Gestational age	Birth weight	Consanguinity	Maternal age	Paternal age	Family history of CHD	Family history of genetic diseases or developmental delay	Obstetric history
2	2.5 months	F	Full term	Normal	–ve	28 y	30 y	–ve	+ ve (3 cousins had mild mental retardation)	–ve
6	5 years	F	Full term	Normal	–ve	35 y	40 y	–ve	+ ve (aunt had global developmental delay)	1st trimester abortion twice
9	15 months	F	Full term	Normal	–ve	27 y	45 y	–ve	–ve	–ve
12	4 months	M	Full term	Normal	–ve	23 y	25 y	–ve	+ ve (aunt had polydactyly)	–ve

Table 8 Clinical and laboratory features of patients with 22q11.2 microdeletion.

No.	Cardiac defect	Extracardiac features	Serum calcium	CBC	Thymus shadow on X-ray
2	PDA	Microcephaly, brain atrophy, narrow palpebral fissure, epicanthus, depressed nasal bridge, anteverted nostrils, microstomia, micrognathia, low set posteriorly rotated ears, small vertical diameter of the ears, recurrent episodes of generalized seizures	Low	Microcytic anemia (Hb 7.3 g/dl)	Normal
6	VSD	Normal mentality, delayed acquisition of motor milestones but eventually normal. Hypertelorism, a broad forehead, malar flattening, epicanthic fold, arched eyebrows, upward slanting palpebral fissure, hypoplastic ala nasi, delayed teething, severe dental caries, micrognathia, attached ear lobule, and posteriorly rotated ears	Low in infancy but eventually normal	Moderate lymphopenia, microcytic anemia (Hb 10.3)	Normal
9	Double outlet right ventricle	Delayed motor and mental milestones. Microcephaly, brain atrophy, recurrent generalized seizures, hypertelorism, slight downward slanting palpebral fissure, hooding of the eyelids, mild parietal bossing, low set ears with attached lobule, broad nasal bridge, umbilical hernia, epigastric hernia, unilateral simian crease, tapering fingers, and camptodactyly of the fifth finger	Low	Moderate lymphopenia	Normal
12	PDA and 2 ASDs	Delayed motor and mental milestones, microcephaly, brain atrophy, agenesis of the corpus callosum, absent septum pellucidum. Weight and length below the 3rd percentiles, anal stenosis, laryngeal web (causing severe obstruction), deep set eyes, triangular face, partial synophrys, epicanthic fold, mild upward slanting palpebral fissure, anteverted nostrils, asymmetric crying face, micrognathia, low set ears, posteriorly rotated ears, skin tag on the right helix, overriding toes (familial)	Normal	Normocytic anemia (Hb 9.5 g/dl)	Normal

PDA = patent ductus arteriosus.

VSD = ventricular septal defect.

ASD = atrial septal defect.

tip of a huge iceberg. Many physicians, if not most of them, overlook non-cardiac congenital abnormalities in these patients. One of our aims was to participate in shedding light on this specific population of patients with syndromic CHD. In this context, we carefully examined all patients referred for cardiac disease with the purpose of finding out any associ-

ated extracardiac anomalies which warrant further evaluation by high resolution karyotyping and FISH.

In our study, chromosomal aberrations were detected in 2 patients (10%) by high resolution banding. One of them had a numerical aberration in the form of trisomy 13 (Patau syndrome) and the other had a structural aberration (46,

Table 9 Prevalence of clinical and laboratory features in patients with 22q11.2 microdeletion.

Features present in	No
100% of cases	
Features present in 75% of cases	Hypocalcemia in infancy, microcephaly, brain atrophy, epicanthus, low set ears, micrognathia, posteriorly rotated ears, and anemia
Features present in 50% of cases	Delayed motor and mental milestones, upward slanting palpebral fissure, recurrent seizures, hypertelorism, attached ear lobule, anteverted nostrils, PDA, and lymphopenia
<i>Features present in 25% of cases</i>	
Growth	Decreased weight and decreased length
Brain	Agnesis of the corpus callosum, absent septum pellucidum
Heart	DORV, VSD, ASD
Face	Asymmetric crying face, malar flattening, broad forehead, deep set eyes, triangular face, partial synophrys
Ocular	Hooding of the eyelids, antimongoloid slant, narrow palpebral fissure, arched eyebrows
Nose	Broad nasal bridge, depressed nasal bridge, hypoplastic ala nasi
Ear	Skin tag on the helix, short vertical diameter of ear
Abdomen	Umbilical hernia, epigastric hernia, anal stenosis
Mouth	Microstomia, dental caries, delayed teeth eruption
Larynx	Laryngeal web
Hands and fingers	Simian crease, camptodactyly of 5th finger, tapering fingers



Figure 1 (A) Photo of patient 3: Patau syndrome (47, XX, +13). (B) Photo of patient 5: 46,XY, add(13)(p13). Patient number 3 (Patau syndrome: Fig. 1A) is a 10 day old full term female newborn. She is the 4th sibling of non-consanguineous marriage. The mother had a history of recurrent spontaneous 1st trimester abortion (three times) and one ectopic pregnancy. There is no family history of CHD, other congenital anomalies, or genetic diseases. Her weight and length are normal but the head circumference is just below the 3rd percentile. She had several dysmorphic features including microcephaly, microphthalmia, narrow palpebral fissure, arched eye brow, epicanthic folds, prominent glabella, broad flat nose, wide nasal bridge, long smooth philtrum, high arched palate, micrognathia, low set ear, loose skin on the nape of the neck, post axial polydactyly, flexed overlapped fingers, and contractures at the elbows. She also had high pitched cry. Echocardiography showed moderate ASD and VSD. Brain CT showed mild brain atrophy. This patient died at the age of 2.5 months due to pneumonia and heart failure. Patient number 5 (Fig. 1B) [with 46,XY, add(13)(p13); Fig. 2] is a 1.5 year old male baby who is the 3rd offspring of a non-consanguineous marriage. The baby was full term with normal birth weight. He had delayed motor milestones but normal mental development. There was no family history of CHD but the first sibling died at the age of 7 months as a result of CNS infection and the parents were told by the physicians that the latter baby had a congenital lung anomaly and immune deficiency. Clinical examination showed failure to thrive with weight, length, and head circumference below the 3rd percentiles. The extracardiac abnormalities included microcephaly, frontal bossing, high arched palate, micrognathia, and low set ears. Echocardiography revealed Tetralogy of Fallot. Total serum calcium was low (6.5 mg/dl). Absolute lymphocytic count was also low for age (2400 ml⁻¹).

XY, add(13)(p13). No chromosome 22 deletion was discovered by high resolution banding.

Many other studies have previously established a chromosomal origin of syndromic CHD. Elsobky et al. [16] found

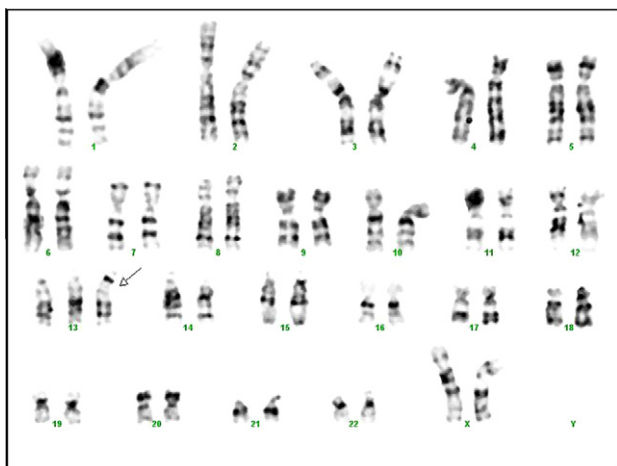


Figure 2 Karyotyping of patient number 3: 47, XX, +13 (Patau syndrome).

chromosomal anomalies, by high resolution banding, in 37% of a group of patients with syndromic CHD, including 3 patients with visible 22q11.2 deletion. Likewise, Soares et al. [17] reported a prevalence of 26.1% for chromosomal anomalies in patients with syndromic CHD.

The 22q11.2 deletion syndrome is one of the most frequently recognized syndromes associated with CHD [18]. In our study, 4 children (20%) were found to have 22q11.2 microdeletion by FISH technique. The prevalence of this microdeletion in patients with syndromic CHD has varied among studies. Consistent with our results, Bartsch et al. [19] reported a prevalence of 19.7% in a series of 295 patients with suspected DG/VCFS. Also, Elsobky et al. [16] detected 22q11.2 microdeletion in 18.6% of patients with syndromic CHD. Other studies reported much lower prevalence. For example, Alikashioglu et al. [20] reported a prevalence of 6.9% for 22q11.2 deletion in Turkish patients with syndromic conotruncal heart diseases. On the other hand, some studies reported higher prevalence rates. One such study is that of Soares et al. [17] who reported a prevalence of 30.4% in a group of patients from Portugal with CHD and other anomalies and patients with a phenotype consistent with 22q11 microdeletion. The discrepancy among these results might be explained in several ways including different ethnic backgrounds [21].

The pattern of congenital heart disease observed in our 22q11.2 deletion patients was unexpected. It included PDA, VSD, ASD, and double outlet right ventricle (DORV). Though VSD is one of the most common cardiac defects in 22q11.2 DS [22], DORV and ASD are among the least common [23,24] and isolated PDA is virtually one of the rare cardiac lesions associated with 22q11.2 DS [25]. And although Tetralogy of Fallot (TOF), is known to be the most common cardiac malformation associated with 22q11.2 DS, occurring in about 26% of cases [23], none of our patients with the microdeletion had this type of cardiac malformation.

How can these intriguing results be explained?

The small number of our study group is a possible explanation and one may expect that a larger series would reveal results more consistent with other larger studies. Another explanation is the relative increase in the prevalence of ASD and PDA (45% and 20% of total patients, respectively) compared with Tetralogy of Fallot (10%) in the study population.

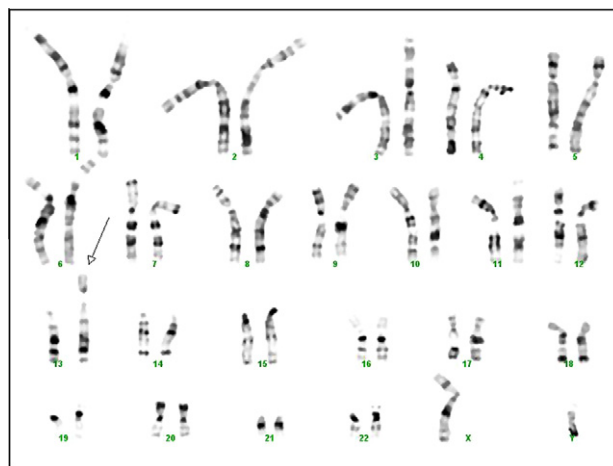


Figure 3 Karyotyping of patient number 5: 46,XY, add (13)(p13).

However, it is logic to think of other factors such as ethnicity. Ethnic differences are known to exist in terms of the relative prevalence of congenital heart diseases [23]. For European patients with the 22q11.2 deletion, TOF with or without pulmonary atresia was found in 27% [24]. However, among Japanese patients with this syndrome, TOF with or without pulmonary atresia was much more common (73%) [26]. The incidences of cardiovascular anomalies among Korean patients with the 22q11.2 deletion are similar to those observed in Japan [25].

Anyhow, until further studies are conducted in this regard, our results speak against the trend adopted by those centers which screen for 22q11.2 deletion only in children with conotruncal heart defects. It is thus prudent, as is the case in other centers, to screen for 22q11.2 deletion among patients with any type of congenital cardiac defects, whether or not conotruncal especially if associated with other anomalies. In our opinion, this may be the most significant conclusion drawn from this small study. We hope to help change the idea stuck to 22q11.2 microdeletion as a syndrome linked only to specific types of congenital heart defects.

In this study, many extracardiac features were found to be associated with 22q11.2 microdeletion, the most frequent among which were hypocalcemia, microcephaly, brain atrophy, epicanthus, low set ears, posteriorly rotated ears, micrognathia, and anemia. The other features are enumerated in Table 8. Most of these features have been reported to be associated with 22q11.2 microdeletion [24,27–32].

Hypocalcemia was found in 3 of our four patients (75%) with microdeletion. This is consistent with other authors who reported the occurrence of hypocalcemia in a substantial proportion of 22q11.2 microdeletion. For example, Kitsiou-Tzeli et al. [33] found a prevalence of 47% for hypocalcemia while Bassett et al. [34] reported a prevalence of 64%.

Seizures in 22q11.2 SD are usually, but not always, due to hypocalcemia and it should be borne in mind that about 7% of cases have unprovoked seizures [35]. Seizures were existent in 2 of our 4 (50%) patients with 22q11.2 deletion. Both of them had the 2 potential causes of seizures, i.e. hypocalcemia and brain atrophy.

Three of our four patients with 22 microdeletion had both microcephaly and cerebral atrophy. This is consistent with Ryan et al. [24] and Kobrynski and Sullivan [36], who reported that



Figure 4 Photos of the 4 patients with microdeletion 22q11.2.

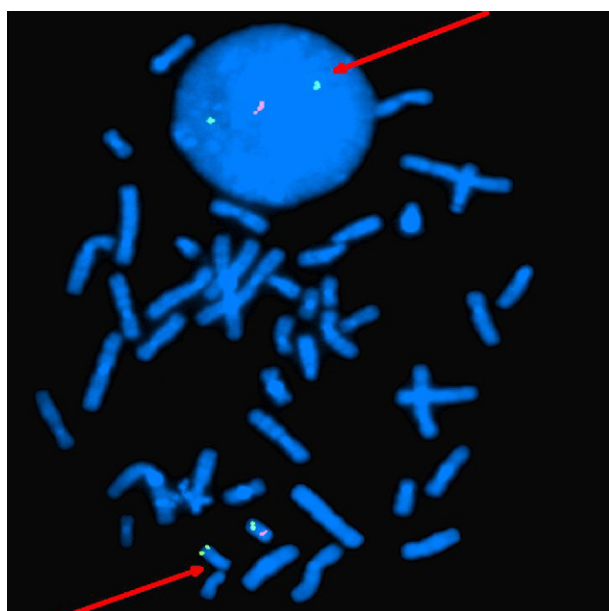


Figure 5 Metaphase and interphase FISH of case 12 showing absence, of the red signal in 1 chromosome 22 denoting microdeletion [46, XY, ish del(22) (q11.2q11.2)(TUPLE1x1), 22q13(ARSAx2)].

cerebral atrophy is among the structural brain abnormalities in 22q11.2 DS. As to microcephaly, Barnea-Goraly et al. [37] and Campbell et al. [38] showed that it occurred in about 50% of 22q11.2 DS patients. Others showed that the prevalence of microcephaly is only 10% [39]. In addition, two of our four patients with 22 microdeletion had delayed motor and mental development. This is consistent with many studies [40].

Laryngeal web was detected in one of our patients with 22 microdeletion (case 12). Laryngeal web had previously been thought to be an uncommon association with 22 microdeletion, but nearly two-thirds of the patients presenting with anterior glottic webs were found to have the deletion. This identifies another subgroup for which screening is appropriate [41].

One of our patients had an anal abnormality in the form of anal stenosis. This is consistent with Ryan et al. [24] who detected anal abnormalities in 2% of their patients with 22q11.2 microdeletion, consisting of anal atresia, imperforate anus, and anteriorly placed anus.

The majority of patients with 22q11.2 deletion syndrome and immune defects exhibit mild to moderate deficits in T-cell counts [42]. In line with this fact, moderate lymphopenia was detected in two of our patients with 22q11.2 microdeletion. They experienced recurrent chest infections and had a normal thymus size on X-ray. The presence of a normal thymic shadow, by X-ray, in our patients with 22 microdeletion is not surprising as, according to Sullivan [43], thymic hypoplasia is not

always directly visualized and diminished T-cell counts are often used as a surrogate. Also, Hussain [44] stated that although chest radiographs may reveal an absence of a thymus shadow, MRI is more reliable for estimating mediastinal thymus size.

Each year brings new information on the phenotypic spectrum of 22q11.2 deletion syndrome [45]. As far as we know, a number of features, which were associated with 22q11.2 DS in our study, have not been previously linked to that syndrome. These included camptodactyly of the 5th finger, simian crease, triangular face, arched eyebrows, broad forehead, deep set eyes, and partial synophrys [46].

Individuals with 22q11DS may have mild phenotypes, so marriage and reproduction are not uncommon [47]. This is particularly true in one of our patients (patient no. 6) whose phenotype is so mild especially after successful open heart surgery for VSD. This makes genetic counseling very important in this case. In the future, 50% of the offspring of this patient are expected to inherit the same deletion. Unfortunately, the offspring are usually more severely affected than their parents from whom they inherited the microdeletion [48]. In addition, this particular patient has a special significance: she had always been deemed, by physicians, a case of isolated CHD based on the normal motor and mental development and the absence of other major congenital anomalies. It was only when she had been examined by experienced dysmorphologists that she transpired to have additional minor dysmorphic features which warranted further genetic evaluation. This clearly stresses the need for incorporating geneticists into the teams evaluating all patients with CHD.

It is concluded that 22q11.2 microdeletion is a very important etiology underlying syndromic CHD. This microdeletion should be looked for, by FISH technique, in all such patients if their conventional karyotyping results are normal especially those with hypocalcemia, microcephaly, brain atrophy, epicanthus, low set ears, posteriorly rotated ears, micrognathia, and anemia. However, the features of the syndrome show marked variability and range from very mild to severe or lethal. Consequently, screening for the 22 microdeletion should be performed in all children with syndromic CHD even if the phenotype is subtle and whether or not the type of CHD is conotruncal.

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