

Microdeletion Study in Children with Selective Congenital Heart Disease; an Iranian Multicenter Study

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Abstract

Objective: Determining the frequency of chromosome 22q11.2 microdeletion in children with congenital cardiac conotruncal abnormalities using Fluorescence in-situ Hybridization (FISH) technique and estimating relation between DiGeorge Syndrome and cardiac conotruncal abnormalities.

Methods: One-hundred and eighty cases (106 Males, 74 Females) with selective congenital heart disease (conotruncal abnormalities) referred to the hospitals affiliated to Tehran University during 2004-2007 were evaluated by pediatric cardiologists. All patients were assessed for chromosome 22q11.2 microdeletion using FISH technique. Consequently, patients with 22q microdeletion were studied for T cell abnormalities.

Findings: Median age of the patients at the time of study was 18 months (3d-16y). The microdeletion of chromosome 22q11.2 was detected in 17 (9.5%) patients with conotruncal abnormalities, including 5 (29.4%) Tetralogy of Fallot plus Supravalvular Pulmonary Stenosis, 4 (23%) Truncus Arteriosus, 5 (29.4%) Pulmonary Arterial Stenosis with Ventricular Septal Defect, 2 (11.8%) CO AO+Interrupted Aortic Arch and one case of Valvular Pulmonary Stenosis. Five of uncorrelated cases had craniofacial dysmorphism.

Conclusion: Chromosome 22q11.2 microdeletion FISH study should be considered in patients with cardiac lesions particularly conotruncal abnormality with or without syndromic problems (craniofacial dysmorphism and developmental delay) to provide an appropriate genetic counseling with more accurate estimation of recurrence risk and ultimately prenatal diagnosis in affected families.

Key Words: FISH; Congenital heart disease; Microdeletion of chromosome 22q11.2; DiGeorge syndrome

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Introduction

Congenital heart disease (CHD) is the most common birth defect and the leading cause of mortality in the first year of life with a prevalence of 1% in live births and 10% in spontaneously aborted fetuses^[1]. CHD is mainly a multifactorial disorder characterized by: a) 90% multifactor disorders, b) 8% chromosomal and single gene disorders and c) 2% environmental teratogens^[2] in majority of cases, CHD occurs as an isolated malformation; however, up to 33% have associated anomalies^[3]. Among chromosomal disorders, chromosome 22q11.2 micro-deletion, is the most common syndrome associated with CHD with estimated prevalence of approximately 1 in 4000 live births^[4,5], 90% of which are sporadic^[6,7]. The various conditions associated with chromosome 22q11.2 microdeletion include DiGeorge anomaly (DG), Velocardio-facial syndrome (VCFS), Conotruncal anomaly face syndrome, isolated conotruncal heart defects and Cayler cardiofacial syndrome^[8-11].

As a result of their common genetic origin and phenotypic overlap, the syndromes were collectively referred to as "CATCH 22" (Cardiac abnormality, Abnormal facies, Thymic hyperplasia, and Cleft palate, Hypocalcemia and chromosome 22q11.2 microdeletion)^[8-12]. The most common cardiovascular anomalies in patients with this syndrome include Tetralogy of Fallot (ToF), Interrupted Aortic Arch, Persistent Truncus Arteriosus and Ventricular Septal Defect (VSD), requiring early clinical intervention^[13]. Several documented studies showed the presence of chromosome 22q11.2 micro-deletion in patients with CHDs ranging (0%-80%)^[11,14,19].

This study investigates the frequency of Chromosome 22q11.2 microdeletion and T cell deficiency in Iranian young population with selective congenital heart disease.

Subjects and Methods

A total of 180 patients (106 males, 74 females) were enrolled in the study. The patients were

selected among children with cardiac abnormalities (excluding those with known numerical chromosome aberrations) referred consecutively from Tehran University affiliated hospitals to the Departments of Pediatric Cardiology of the Children's Medical Center and Imam Khomeini Hospital over a four year period (2004-2007). Median age of the patients at the time of study was 18 months (The age ranged from 3 days to 16 years). All children received a general clinical pediatric examination together with cardiological and genetic evaluation by pediatric cardiologists and a clinical geneticist. The clinical data including weight, height, type of CHD, presence of dysmorphic features, extra cardiac malformations (if any) and psychomotor development were also ascertained.

Chromosomal analysis was then carried out on peripheral blood lymphocyte culture using standard protocol with suitable modifications (Sea bright 1971)^[19]. G-banded metaphase chromosomes were then screened at 450 band level. The chromosomes were analyzed according to the International System for Human Cytogenetic Nomenclature (ISCN 1995)^[20]. Peripheral venous blood of each patient was used for Fluorescence in Situ Hybridization (FISH) analysis as previously described^[15,16]. Briefly, slides were prepared using interphase cells spotted on to cleaned microscope slide, then immersed in 2 x SSC (3 mol/l NaCl, 3 mol/l Na₃-citrate), pH 7.0 for two minutes each and dehydrated in an ethanol series (70%, 85%, 100%) for two minutes each. Then Pre- and post-hybridization FISH was performed using specific probes (DiGeorge/VCFS TUPLE1 Cytocell) based on the protocol recommended by the manufacturer.

FISH images were captured using a fluorescent microscope (Leica CW 4000) equipped with a CCD camera and FISH analysis software (Leica). Subsequently, patients with chromosome 22q11.2 micro-deletion were studied for T cell abnormalities. Patients' white cell count, lymphocyte count, T cell numbers, flowcytometry and clinical history of infections were reviewed.

Table 1: Number of defects detected by echocardiography

Echo	Frequency (%)
PA+ VSD± PDA	53 (29.6)
ARSA	4 (2.2)
PS	3 (1.7)
CO AO± VSD	7 (3.8)
PS+ VSD	13 (7.2)
TrA	18 (10.0)
ToF± SUPRA VALVAR PS	57 (31.8)
COAO+ IAA	10 (5.5)
AS+ BAV+ VSD	4 (2.2)
VSD+ PH	10 (5.5)
PS+ AS	1 (0.5)
Total	180 (100.0)

PA= Pulmonary Atresia, VSD= Ventricular Septal Defect, TrA= Truncus Arteriosus, PDA= Patent Ductus Arteriosus, ARSA= Aberant Right Subclavian Aortic Arch, AS=Aortic Stenosis, IAA= Interrupted Aortic Arch, CO.AO= Coarctation of Aorta, PS= Pulmonary Stenosis, TOF= Tetralogy Of Fallot, PH= Pulmonary Hypertention

Findings

One hundred eighty patients with conotruncal cardiac abnormalities aged 3 days - 16 years (Median 18 months) including 106 (59%) males and 74 (41%) females, were enrolled in this study. The distribution of different congenital heart diseases among these patients is shown in Table 1. The most common congenital heart defect was Tetralogy of Fallot (ToF) accompanied with supravalvular pulmonary stenosis. FISH analysis revealed chromosome 22q

microdeletion in 17 (9.5%) patients. Among the FISH positive patients, 5 (29.4%) ToF plus supravalvular pulmonary stenosis, 5 (29.4%) had Pulmonary Atresia (PA) with ventricular septal defect (VSD), 4 (23.5%) had truncus arteriosus (TA). More details are shown in Table 2. According to the clinical characteristics of the patients, in FISH positive group, 4 (23.5%) had developmental delay, 5 (29.6%) had craniofacial dysmorphism and two (12%) had positive family history. Nine out of 17 FISH positive patients were male and 8 female. The spectrum of heart defects, and

Table2: Patients who had positive FISH test

ECHO - FISH Positive CROSSTABULATION	Frequency (%)
PA+ VSD± PDA	5 (29.4)
TrA	4 (23.5)
ToF± Supra Valvar PS	5 (29.4)
CO Ao+ IAA	2 (11.8)
PS	1 (5.9)
Total	17 (100.0)

PA= Pulmonary Atresia, VSD= Ventricular Septal Defect, TrA= Truncus Arteriosus, PDA= Patent Ductus Arteriosus, IAA= Interrupted Aortic Arch, CO.AO= Coarctation of Aorta, PS= Pulmonary Stenosis, TOF= Tetralogy Of Fallot

other related findings in FISH positive patients are shown in table 3.

Among 17 FISH positive patients, only 2 patients had mild thymic aplasia diagnostic of DiGeorge/VCFS syndrome. The remaining 15 patients had no evidence of diminished T cells.

Discussion

The present study was performed to investigate the 22q11.2 microdeletion among Iranian children with congenital heart disease (CHD). FISH analysis using TUPLE 1 (Cytocell) probe on all the 180 CHD patients involved in the study revealed microdeletion in 9.5% (17) of the cases.

In a population-based study of 255849 births Lorenzo et al^[21] identified 43 children

with 22q11.2 deletion. The overall prevalence was 1 in 5950 births. They concluded that such population-based studies would be useful to medical professionals and policy makers in planning for the optimal care of people with the 22q11.2 deletion^[21,22].

Tobias et al^[23] studied on 551 children with cardiac anomalies using FISH analysis and revealed 12% of cases with 22q11.2 microdeletion. In another study McDonald-McGinn et al^[24] investigated the phenotype of the 22q11.2 deletion individuals, 30 individuals with a 22q11.2 deletion were identified following the diagnosis in a relative. Sixty percent of the patients had no visceral anomalies; this showed the importance of deletion testing in parents of affected probands. The authors emphasized the importance of broadening the index of suspicion in order to provide appropriate

Table 3: Characteristics of cases with positive finding and FISH positive

Number	Sex	Convulsion	Family history	Craniofacial Dysmorphism	Developmental Delay	Echocardiography Or Angiography	Chest X-Ray	FISH Test	T-cell Deficiency
1	F	yes	no	no	yes	ToF ± Supra Valvar PS	normal	Positive	+
2	F	no	yes	yes	yes	TrA	normal	Positive	-
3	F	no	no	no	no	PA + VSD	normal	Positive	-
4	F	no	no	no	no	PS	normal	Positive	-
5	F	no	no	yes	yes	ToF ± Supra Valvar PS	normal	Positive	-
6	F	no	no	no	no	TrA	normal	Positive	-
7	F	no	no	no	no	PA+VSD	normal	Positive	-
8	F	no	no	no	no	TOF	normal	Positive	-
9	M	no	no	no	no	CO AO	normal	Positive	-
10	M	no	yes	no	no	TrA	normal	Positive	-
11	M	no	no	yes	yes	CO AO+ IAA	Abnormal	Positive	+
12	M	no	no	no	no	PA+VSD	normal	Positive	-
13	M	no	no	no	no	ToF ± Supra Valvar PS	normal	Positive	-
14	M	no	no	yes	no	PA+VSD	normal	Positive	-
15	M	no	no	no	no	PA+VSD	normal	Positive	-
16	M	no	no	no	no	TrA	normal	Positive	-
17	M	no	no	yes	no	TOF	normal	Positive	-

PA= Pulmonary Atresia, VSD= Ventricular Septal Defect, TrA= Truncus Arteriosus, IAA= Interrupted Aortic Arch, CO.AO= Coarctation of Aorta, PS= Pulmonary Stenosis, TOF= Tetralogy Of Fallot

recurrence risk assessment, cognitive remediation, and medical management. Studies by Fokstuen et al^[26] with a sample size of 110 non-selective (syndromic+ non-syndromic) CHD using the D22S75 DiGeorge chromosome region probe (FISH), showed 9/51 (17.6%) syndromic patients. Five were of maternal origin and four of paternal origin. None of the 59 patients with isolated congenital cardiac defect had a 22q11.2 deletion. Borgmann et al^[25] concluded that testing for the 22q11.2 microdeletion is clearly indicated in cases when even mild extracardiac abnormalities are present, particularly in very young infants.

In another study carried out by Gawde et al^[27] with a sample size of 105 prospective cases which included 6 families with isolated, non-syndromic cardiac defects, showed only 5.71% chromosome 22q11.2 microdeletion. They suggested that testing for chromosome 22q microdeletion in isolated non-syndromic patients using FISH technique is mandatory even when mild/unspecific extracardiac abnormalities are seen in the patients.

At the present FISH study the microdeletion of 22q11.2 was detected in 9.5% of patients. Five out of 22 syndromic patients (DGS/VCFS indices) had 22q11 micro-deletion. Ryan et al^[14] presented clinical data on 558 patients with deletions within the DiGeorge syndrome critical region of chromosome 22q11.2, and immunological problems were very uncommon. They reported only 4 major immune function abnormalities and 15 cases with clinical history of infection. Sullivan^[28] reported low T-cell numbers in 75% to 80% of infants who had chromosome 22q11.2 deletion syndrome. In most cases this problem was mild or moderate and did not have an impact on the procedures. However, it is not known whether postoperative infections are increased in this population. Less than 1% of patients who have the deletion lack T cells, but these patients represent a special category at the time of cardiac surgery and require protection from infection and blood products. Blood products can induce graft-versus-host disease in patients without T cells, and graft-versus-host disease from transfusions almost

always is fatal. At the present study 2% of all patients and 12% of patients with microdeletion 22q11.2 had mild to moderate T cell/thymus abnormality.

Children who have chromosome 22q11.2 deletion syndrome and a mild to moderate decrement in the T-cell count have largely normal immunoglobulin levels and T-cell proliferative responses. T-cell numbers decline with age in all children, but the decline in patients who have chromosome 22q11.2 deletion syndrome seems to be slower. In fact, adults who have chromosome 22q11.2 deletion syndrome have normal T-cell numbers for the most part. The slower age-determined decline in T-cell numbers in patients compared with controls is caused by the homeostatic proliferation of existing T cells^[28].

Conclusion

Association between CHDs and chromosome 22q11.2 microdeletions suggests that FISH analysis should be performed in CHD patients even when there are mild, unspecific extra cardiac anomalies. Due to the wide range and extremely variable expression of extra cardiac anomalies, a careful general clinical examination and gentle work up, together with genetic counseling in patients with or without syndromic CHD finding are compulsory. In cases with chromosome 22q11.2 microdeletion, both parents must be studied and an appropriate genetic counseling must also be offered to provide more accurate estimation of recurrence and possibility of prenatal diagnosis.

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References

1. Kaneko Y, Chevtchik O, Mohl W, et al. Recent advances in genetics of cardiac diseases. *Heart View* 1999;1(5):152-62.
2. Larson RS, Butler MG: Use of fluorescence in situ hybridization (FISH) in the diagnosis of DiGeorge sequence and related disease diagnosis. *Mol Pathol.* 1995;4(4):274-8.
3. Rauch A, Hofbeck M, Leipold G, et al. Incidence and significance of 22q11 hemizigosity in patients with interrupted aortic arch. *Am J Med Genet.* 1998;78(4): 322-31.
4. Marino B, Digilio MC, Persiani M, et al. Deletion 22q11 in patients with interrupted aortic arch. *AM J Cardiol.* 1999;84(3):360-1.
5. Oh DC, Min JY, Lee MH, et al. Prenatal diagnosis of tetralogy of Fallot associated with chromosome 22q11. 2q11 del. *J Korean Med Sci.* 2002;17(1):125-8.
6. Aubrey M, Demczuk S, Desmaze C, et al. Isolation of a zinc finger Gene consistently deleted in DiGeorge syndrome. *Human Molecular Genet.* 1993;2(10):1583-7.
7. Lewin MB, Lindsay EA, Jurecic V, et al. A genetic etiology for interruption of the aortic arch type B. *Am J Cardiol.* 1997; 80(4):493-7.
8. Ardinger HH, Ardinger RH. Clinical presentation of velo-cardio-facial syndrome. *Progr Pediatr Cardiol.* 2002; 15(2):93-7.
9. Eapen RS, Eidem BW. Echocardiographic assessment of velocardiofacial syndrome. *Progr Pediatr Cardiol.* 2002; 15(2):125-33.
10. Jones CH, Gowronski MJ. The genetic 22q11.2 deletion syndrome. *Progr Pediatr Cardiol.* 2002;15(2):99-101.
11. Sullivan KE. Immunologic issue in VCFS/Chromosome 22q11.2 deletion syndrome. *Progr Pediatr Cardiol.* 2002; 15(2):103-8.
12. Marino B, Digilio CM, Toscana A. Common aortic trunk, DiGeorge syndrome and microdeletion 22q11. *Pediatr Cardiol.* 2002;15(1):9-17.
13. Earing M, Ackerman MJ, Driscoll DJ. Cardiac phenotype in the chromosome 22q11.2 microdeletion syndrome. *Progr Pediatr Cardiol.* 2002;15(2):119-23.
14. Ryan AK, Goodship JA, Wilson DI, et al. Spectrum of clinical features associated with interstitial chromosome 22q11 deletions: a European collaborative study. *J Med Genet.* 1997;34(10):798-804.
15. Ghaffari SR, Boyd E, Connor JM, et al. Mosaic supernumerary ring chromosome 19 identified by comparative genomic hybridization. *J Med Genet.* 1998;35(10):836-40.
16. Ghaffari SR, Boyd E, Tolmie JL, et al. A new strategy for cryptic telomeric translocation screening in patients with idiopathic mental retardation. *J Med Genet.* 1998;35(3):225-33.
17. McDonald-McGinn DM, Driscoll DA, Goldmuntz E, et al. Velopharyngeal incompetence diagnosed in a series of cardiac patients prompted by the findings of a 22q11.2 deletion. *Am J Hum Genet.* 1994;55(1):35.
18. McElhinney DB, Driscoll DA, Levin ER, et al. Chromosome 22q11 deletion in patients with ventricular septal defect: frequency and associated cardiovascular anomalies. *Pediatrics.* 2003;112(6 pt 1): e472.
19. Pagotto LT, Hawkins JA, Heidi Pollard T, et al. Management of Cardiac anomalies associated with velo cardiofacial syndrome. *Progr Pediatric Cardiol.* 2002; 15(2):135-44.
20. Iserin L, de Lonlay P, Viot G, et al. Prevalence of the microdeletion 22q11 in newborn infants with congenital conotruncal cardiac anomalies. *Eur J Pediatr.* 1998;157(11):881-4.

21. Botto LD, May K, Fernhoff PM, et al. A population-based study of the 22q11.2 deletion: phenotype, incidence and contribution to major birth defects in the population. *Pediatrics*. 2003; 112(1 pt 1):101-7.
22. Portnoi MF, Joy N, Gonzales M, et al. Prenatal diagnosis by FISH of a 22q11 deletion in two families. *Med Genet*. 1998;35(2):165-8.
23. Tobias ES, Morrison N, Whiteford ML, et al. Towards earlier diagnosis of 22q11 deletions. *Arch Dis Child*. 1999;81(6): 513-4.
24. McDonald-McGinn DM, Tonnesen MK, Laufer-Cahana A, et al. Phenotype of the 22q11.2 deletion in individuals identified through an affected relative: cast a wide FISHing net. *Genet Med*. 2001;3(1):23-9.
25. Borgmann S, Luhmer I, Arslan-Kirchner M, et al. A search for chromosome 22q11.2 deletions in a series of 176 consecutively catheterized patients: no evidence for deletions in non-syndromic patients. *Eur J Pediatr*. 1999;158(12): 958-63.
26. Fokstuen S, Arbenz U, Artan S, et al. 22q11.2 deletions in a series of patients with non-selective congenital heart defects: incidence, type of defects and parental origin. *Clin Genet* 1998;53(1): 63-9.
27. Gawde H, Patel ZM, Khatkhatey MI, et al. Chromosome 22 microdeletion by F.I.S.H. in isolated congenital heart disease. *Indian J Pediatr*. 2006;73(10):885-8.
28. Sullivan KE. Chromosome 22q11.2 deletion syndrome: DiGeorge syndrome/Velocardiofacial syndrome, *Immunol Allergy Clin North Am*. 2008;28(2):353-66.