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Screening for TBX1 Gene in Children With or Without Microdeletion of Chromosome 22q11 and Conotruncal Defect

Alka Anilkumar, MSc,[1] Mahesh Kappanayil, FNB,[2] Sheela Nampoothiri, DNB, MSc,[3] M.V. Thampi, MSc,[1] D.M. Vasudevan, MD, FRCPath,[4] R. Krishna Kumar, MD, DM, FACC, FAHA[2]

([1]Department of Human Cytogenetics, [2]Department of Paediatric Cardiology, [3]Department of Paediatric Genetics, [4]School of Medicine, Amrita Institute of Medical Sciences and Research Centre, Kochi, India)

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Abstract

Objective: In order to understand the role of the TBX1 gene in humans, Indian children with or without a microdeletion of chromosome 22q11 and conotruncal defects were screened by fluorescent in situ hybridization (FISH) using a TBX1 commercial probe.

Methods: All patients were tested with commercial FISH probes on chromosome 22q11. Besides these, the deletion of the distal arm of chromosome 10 was analyzed, and phenotypic features were also taken into consideration.

Results: A comparable result showing 22q11 deletion was obtained using the 3 probes (TBX1, TUPLE1, and N25) from the chromosome 22q11 region.

Conclusion: The study confirms the role of contiguous genes including TBX1 in the pathogenesis of conotruncal defects. It also indicates that in a routine clinical practice either of the FISH probes (ie, TUPLE1, N25 or TBX1) can be used to detect 22q11 deletion. Such genetic testing is important, since patients with 22q11 deletion need early medical intervention based on associated symptoms.

Keywords: 22q11.2 deletion, conotruncal defects, TBX1

It is well established that genetics play a significant role in the etiology of 22q11 deletion syndrome. An interstitial deletion of chromosome 22q11 is mainly characterized by conotruncal heart defect, cleft palate, facial dysmorphism, immune disorders, developmental and behavioral problems, and psychiatric disorders, mainly Schizophrenia. The chromosome 22q11.2 region contains nearly 30 well-defined genes including TBX1. Transgenic complementation and gene targeting studies showed that loss of the Tbx1 gene is responsible for cardiovascular abnormalities in mice.[1,2] In approximately 94% of cases, the 22q11 deletion can be identified by fluorescence in situ hybridization (FISH) using TUPLE1 probe;[3] however, it fails to detect deletions of the TBX1 locus within the 22q11.2 region. In order to understand the role of the TBX1 gene in humans, 22 children with or without microdeletion of chromosome 22q11 and conotruncal defect were tested by TBX1 probe.

Materials and Methods

The study was carried out in Indian children up to 2 years of age. The blood was drawn with the informed consent from parents of affected children. The institutional review board had approved the study.

The 22 patients with conotruncal defects were comprised of 2 groups: Group I had 10 patients with deletion and Group II had 12 patients without deletion for TUPLE1 locus on chromosome 22q11. These patients were screened for DiGeorge critical region by FISH technique using commercial probe TBX1 (Cytocell, Cambridge, U.K.) and N25 (Vysis, Abbott Molecular, Abbott Park, IL) on chromosome 22q11. The TBX1 probe (213Kb) can detect deletion of the TBX1 locus on 22q11.2 and contains a SHANK3 gene as a control that binds at 22q13.3. In addition, they were tested with a DGCRII probe (Cytocell), which identifies the deletion of the distal half of BRUNOL3 on the short arm of chromosome 10. A total of 25 GTG (G bands using Trypsin and Giemsa) banded chromosomes were also analyzed for each patient. The phenotypic features were evaluated by a pediatric clinical geneticist. In addition, serum thyroid function test was completed in all patients.

Results

All patients showed normal karyotype (**Image 1**) with conventional cytogenetic technique. In the cases of children tested with multiple probes, all 10 patients from Group I with a deletion of the TUPLE1 region were also found to have a deletion for TBX1 (**Image 2**) and N25 loci on chromosome 22q11. In Group II, all 12 patients without a deletion for TUPLE1 locus showed normal FISH signals with TBX1 and N25 probe. None of the 22 patients were found to have a deletion of DGCRII on chromosome 10p13-14. The frequency of different extracardiac features in 22 patients with deletion (n=10) and without deletion (n=12) is shown in **Table 1**. None of the patients had hypothyroidism.

Corresponding Author

Alka Anilkumar, MSc
alkaanilkumar@gmail.com

Abbreviations

FISH, fluorescent in situ hybridization; VCFS, Velo-Cardio-Facial Syndrome; CGH, comparative genomic hybridization; MLPA, multiplex ligation-dependent probe amplification; qPCR, quantitative real-time polymerase chain reaction

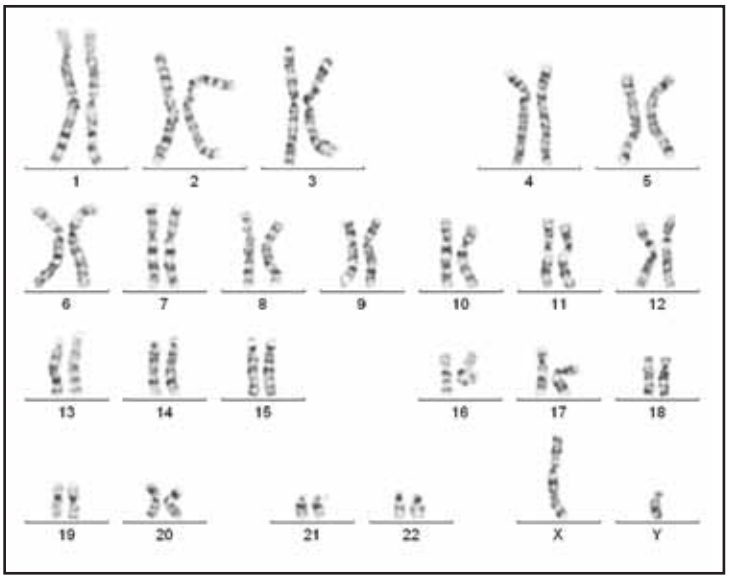

Image 1_Cytogenetic analysis showing a normal GTG banded karyotype.

Table 1_Extracardiac Features in Patients Tested With TBX1, TUPLE1, and N25 Probes

Extracardiac Features	With Deletion No. (%), n=10	Without Deletion No. (%), n=12
Developmental delay	4 (40%)	3 (25%)
Microcephaly	6 (60%)	3 (25%)
Dysplastic flared pinna	8 (80%)	7 (58%)
Posteriorly placed pinna	2 (20%)	1 (8%)
Asymmetric ears	2 (20%)	2 (17%)
Hypertelorism/telecanthus	4 (40%)	7 (58%)
Short palpebral fissures	3 (30%)	3 (25%)
Abnormal eye slant	0 (0%)	1 (8%)
Hypoplastic ala nasi	3 (30%)	1 (8%)
Bulbous nasal tip	9 (90%)	8 (67%)
Prominent hooked nose	1 (10%)	0 (0%)
Facial asymmetry	2 (20%)	0 (0%)
Micrognathia	7 (70%)	6 (50%)
Microstomia	2 (20%)	1 (8%)
High arched palate	4 (40%)	4 (33%)
Cleft palate	1 (10%)	0 (0%)
Thin long fingers	9 (90%)	7 (58%)
Seizures	0 (0%)	0 (0%)
Mental retardation	2 (20%)	1 (8%)
Hypocalcemia	4 (40%)	1 (8%)

Discussion

During embryogenesis the TBX1 gene may interact with multiple genes involved in the development of pharyngeal arches and pouches forming the heart, head, and neck.[4,5,6] Although in mice the Tbx1 gene is found to play a key role in the development of the heart,[7] in about 25% of deleted patients with Velo-Cardio-Facial Syndrome (VCFS), no heart defect was noticed.[8] Our study shows that in Group I, all 10 patients with conotruncal defects had a deletion of TBX1, TUPLE1, and N25 loci. Hence, it is likely that genetic interaction of more than 1 gene including TBX1 is the cause for 22q11 deletion syndrome,[8] with a wide range of phenotypic variabilities. In cases of non-deleted patients with DiGeorge or VCFS a mutation of TBX1 is rarely found, the cause of which is unknown.[9] In the present study children without the deletion of the TUPLE probe were also not without the TBX1 region, indicating that the etiology of conotruncal defects is multifactorial.

The routine diagnostic procedure to detect such submicroscopic deletion is FISH, which is easily accessible. Although FISH fails to detect atypical deletion found in 5% of cases with 22q11 deletion syndrome, such deletions are rarely found in conotruncal defect.[3] The more advanced molecular techniques, like comparative genomic hybridization (CGH), multiplex ligation-dependent probe amplification (MLPA), and quantitative real-time polymerase chain reaction (qPCR) can be used for the accurate detection of genomic changes but are available at limited centers. Therefore, we believe diagnosis of the chromosome

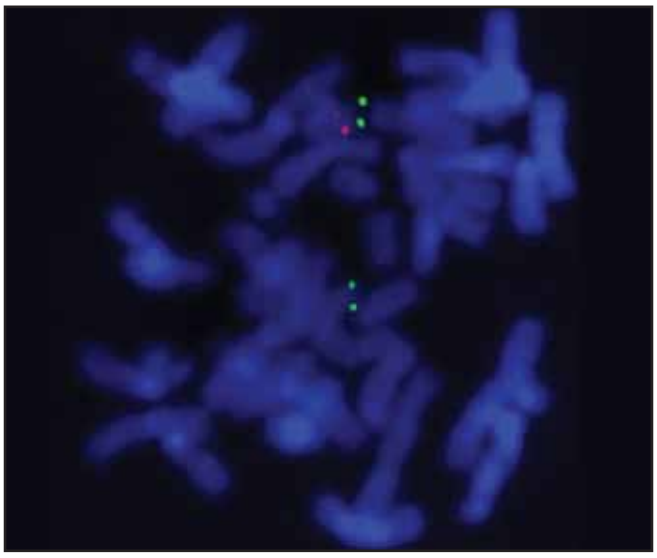

Image 2_Fluorescent in situ hybridization using a TBX1 probe shows chromosome 22q11 microdeletion (absence of a red spot indicates monosomy for TBX1 gene and 2 green signals specify control spots) on metaphase spread.

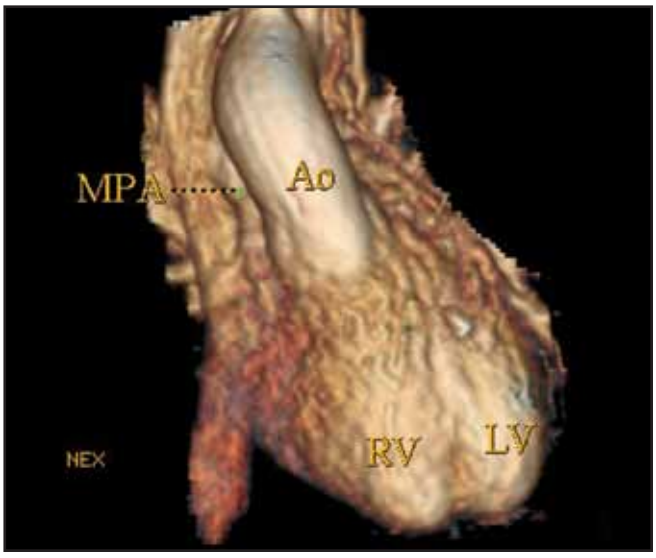

Image 3_Gadolinium enhanced MR Angiography 3-D reconstruction of a heart with double outlet right ventricle—an example of a conotruncal defect.

22 deletion by FISH technique is important in conotruncal defect, especially in centers lacking the advanced techniques.

The advantage of using TBX1 probe is that it can detect deletion of the TBX1 locus, which can be missed by routine TUPLE1 or N25 probes. This was noticed in an infant with pulmonary atresia, ventricular septal defect, facial dysmorphia, neonatal hypocalcemia, and his affected mother with learning disabilities, facial dysmorphia, and hypernasal speech.[10]

The frequency of extracardiac features including dysplastic flared pinna, bulbous nasal tip, long thin fingers, and micrognathia were more frequent in children with 22q11 deletion than those without deletion. The etiology for this may be a developmental defect as a result of deletion of contiguous genes. Our series of 22 patients showed no deletion of chromosome 10p13-14, consistent to findings in 100 patients with conotruncal defects,[11] representing a low incidence of deletion of DGCRII in conotruncal malformations. Hypothyroidism in patients with 22q11 deletion may be due to Tbx1 inactivation resulting in thyroid dysgenesis,[12] but none of the patients deleted for TBX1 gene had hypothyroidism in the present study.

The deletion of chromosome 22q11 using 3 commercial probes (N25, TUPLE1, and TBX1) helps in characterizing the extent of the deletion and confirms the role of the contiguous gene in the pathogenesis of conotruncal defects (**Image 3**). Since multiple genes are involved in the etiology of this syndrome, in routine clinical practice any of the FISH probes (ie, TUPLE1, N25, or TBX1) can be used to detect 22q11 deletion in conotruncal defects. The results of such genetic testing is important for clinical decision makers, since patients with 22q11 deletion need early medical intervention based on associated symptoms. However, a more detailed study on the mechanism of interaction between multiple genes is important in order to understand the exact cause for the 22q11 deletion syndrome. LM

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