Investigation of *TBX1* gene deletion in Iranian children with 22q11.2 deletion syndrome: correlation with conotruncal heart defects

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ABSTRACT

Background DiGeorge syndrome (DGS) is the result of a microdeletion in chromosome 22q11.2 in over 90% of cases. DGS is the second most frequent syndrome after Down syndrome and has an incidence of 1/4000 births. Unequal crossover between low-copy repeats, on the proximal part of the long arm of chromosome 22, usually results in a 3 Mb deletion in one of the chromosome 22 and a reciprocal and similarly sized duplication on the other one. Several studies have indicated that *TBX1* (T-box 1) haploinsufficiency is responsible for many of the phenotypic traits of 22q11.2 deletion syndrome. Conotruncal heart defects (CTDs) are present in 75–85% of patients with 22q11.2 deletion syndrome in Western countries.

Methods Among 78 patients fulfilling the criteria for DGS diagnosed by the fluorescence in situ hybridisation test, 24 had 22q11.2 deletion. Screening for *TBX1* gene deletion was performed by multiplex ligation-dependent probe amplification (MLPA).

Results Our results revealed that of 24 patients with *TBX1* gene deletion, 12 had CTDs while 12 did not show any heart defects.

Conclusions Our findings indicate that other genes or gene interactions may play a role in penetrance or the severity of heart disease among patients with DGS.

INTRODUCTION

DiGeorge syndrome (DGS) and velocardiofacial syndrome (VCFS) are genetic disorders affecting pharyngeal and neurobehavioural development that result in congenital heart defects (CHDs), velopharyngeal insufficiency, hypoparathyroidism, thymic aplasia or hypoplasia, craniofacial dysmorphism, learning difficulties and psychiatric disorders.[1] Usually 15–20% of CHDs are conotruncal heart defects (CTDs). CTDs are most commonly associated with 22q11 deletion syndrome and have a structure similar to that of ventricular outflow tract defects. They include tetralogy of Fallot (TOF), pulmonary atresia with ventricular septal defect (PA/VSD), double outlet right ventricle, transposition of the great arteries, persistent truncus arteriosus and interrupted aortic arch.[2] Low-copy repeats (LCRs) on 22q11 have been suggested to mediate non-allelic homologous recombination, resulting in 22q rearrangement.[3] Unequal crossover between LCRs usually results in a 3 Mb deletion in one of the chromosome 22 and a reciprocal and similarly sized duplication on the other one.[4] The four main blocks of chromosome 22q11-specific repeat sequences are LCR units A–D. These LCRs are 100–400 kb long, the two largest being the most proximal (LCR-A) and most distal (LCR-D) units, which occur at the ends of the typical 3 Mb deletion. These and the other rearrangements involving 22q11 as well as other repeat units are variously found at the ends of smaller atypical deletions.[5] Over 30 genes map within the commonly deleted/duplicated 3 Mb region.[6 7] Most 22q11 deletion syndrome patients (95%) have a 3 Mb interstitial deletion with similar endpoints—the typically deleted region (TDR)—and a minority have a somewhat smaller (1.5–2 Mb) deletion.[8] Although the majority of patients with 22q11 deletion have 3 Mb deletions,[4] most attention has been focused on the 1.5 Mb region between the two most proximal LCRs. Furthermore, the symptoms observed in patients with this deletion are indistinguishable from those seen in patients with the larger deletion. Thus, it is presumed that this region is fundamental to the phenotype.[9]

However, several studies indicate that the haploinsufficiency of *TBX1*, which is the most likely gene responsible for 22q11 deletion syndrome, causes many of the phenotypic traits of the syndrome.[6 10] *TBX1* has emerged as a central player in the coordinated formation of organs and tissues derived from the pharyngeal apparatus and the adjacent secondary heart field from which the cardiac outflow tract derives. Several mutations in the *TBX1* gene have been found in children affected by VCFS.[11–13] Other studies have found rare variants with uncertain clinical significance. These variants are not present in the normal population and do not segregate with the phenotypic features of the syndrome. However, this might be due to lack of evidence or the unavailability of functional information.[14 15] In the present study, we screened for *TBX1* gene deletion in 24 Iranian patients with clinical features of 22q11.2 deletion syndrome using multiplex ligation-dependent probe amplification (MLPA).

MATERIAL AND METHODS

Of 78 patients fulfilling the criteria for DGS, 24 diagnosed with 22q11.2 deletion syndrome by the fluorescence in situ hybridisation test (FISH) were included in this study, which was performed in the Genetic Laboratory of Alzahra University Hospital, Isfahan, Iran. The study was approved by the local ethics committee. The patients or their parents were informed of the aims of the study and their consent was obtained before genetics analysis. Echocardiography reports were obtained for all

patients and MLPA was carried out. A set of MLPA probes to screen copy number changes in the 22q11.2 region was developed according to the procedures described in the literature.[16] MLPA analysis was carried out using the SALSA MLPA kit (P250-B1, MRC-Holland) according to the manufacturer's instructions. The P250-B1 kit contains PCR primers for 48 loci with amplification products between 130 and 487 nucleotides and has two probes for the *TBX1* gene. Most 22q11 DiGeorge deletions (87%) start at LCR22-A and end at LCR22-D.[4] The *TBX1* gene is located in the LCR22-A region, which has an exceptionally high GC content.

For MLPA, the ligation reaction was performed using 200 ng of target DNA as follows: (1) denaturation at 98°C for 5 min; (2) hybridisation with the SALSA MLPA kit P250-B1 at 60°C for 16 h; (3) ligation by Ligase-65 mix at 54°C for 15 min; and (4) ligase inactivation by incubation at 98°C for 5 min. Finally, multiplex PCR was performed using the specific SALSA FAM PCR primers, dNTPs, PCR buffer and polymerase for 30 cycles (95°C for 30 s, 60°C for 30 s, and 72°C for 1 min). The fragments were analysed on a 3100 capillary sequencer (Applied Biosystems) with a 36-cm capillary array and POP-4 polymer (Applied Biosystems) by mixing with 0.2 μL of the GeneScan 500 ROX Size Standard (Applied Biosystems) and 10 μL of HiDi formamide (Applied Biosystems). The results (size and the peak area) were analysed using GeneMarker software.

RESULTS

MLPA screening identified a loss of DNA dosage on chromosome 22q11 in the 24 patients with FISH-confirmed 22q11.2 deletion included in this study (table 1). Of these 24 patients, 19 had an identical large deletion of about 3 Mb (LCR22-A to LCR22-D), two had a 2.3 Mb deletion (LCR22-A to LCR22-C), two had a 1.5 Mb deletion (LCR22-A to LCR22-B) and one had a deletion from LCR22-A to the end of the *HIC2* gene which is located in the first LCR-D unit. Twelve patients with deletions had cardiac abnormalities, including six patients with atrial septum defect (ASD)/VSD, two with TOF, two with mitral valve prolapse (MVP)/VSD and two with VSD/patent foramen ovale (PFO).

Table 1 Results of the clinical and molecular analysis of 24 Iranian children with 22q11.2 deletion syndrome

Patient no.	Age	Cardiac defect	Karyotype	Deletion region
1	11	TOF	46,XY	LCR A–C
2	7	N	46,XX	LCR A–D
3	3	ASD/VSD	46,XY	LCR A–D
4	17	N	46,XY	LCR A–D
5	8	N	46,XY	LCR A–D
6	9	N	46,XX	LCR A–D
7	5	ASD/VSD	46,XX	LCR A–B
8	1.5	ASD/VSD	46,XY	LCR A–D
9	12	N	46,XX	LCR A–D
10	18	MVP/VSD	46,XY	LCR A–D
11	7	N	46,XX	LCR A–D
12	18	VSD/PFO	46,XY	LCR A–D
13	4	ASD/VSD	46,XX	LCR A–B
14	2.5	N	46,XX	LCR A–D
15	3	N	46,XY	LCR A–D
16	6	TOF	46,XX	LCR A–C
17	2	N	46,XX	LCR A–D
18	8	N	46,XY	LCR A–D
19	6	ASD/VSD	46,XX	LCR A–D
20	9	N	46,XX	LCR A–D
21	7	MVP/VSD	46,XX	LCR A–D
22	3	VSD/PFO	46,XY	LCR A–D
23	4	ASD/VSD	46,XX	LCR A–C+*HIC2*
24	12	N	46,XY	LCR A–D

ASD, atrial septum defect; LCR, low-copy repeats; MVP, mitral valve prolapse; N, normal; PFO, patent foramen ovale; TOF, tetralogy of Fallot; VSD, ventricular septum defect.

DISCUSSION

TBX1 mutations cause a dramatic and yet relatively specific pharyngeal phenotype which includes craniofacial abnormalities, cleft palate, thymic and parathyroid defects, and some of the more common cardiovascular defects.[17] TBX1 haploinsufficiency is thought to be responsible for many of the phenotypic traits of DGS/VCFS.[5] TBX1 gene mutations have been detected in some patients with DGS/VCFS features who do not exhibit 22q11.2 deletion.[18] In our study, 12 of the 24 patients with CTDs had deletions in the commonly deleted region, suggesting involvement of the *TBX1* gene. Of these patients, 50% (6/12) had VSD/ASD, 16.6% (2/12) had TOF, 16.6% (2/12) had MVP/VSD and 16.6% (2/12) had VSD/PFO. This result is in line with the findings of previous studies which showed a high prevalence of the deletion in patients with ASD/VSD and TOF.[18] Despite the fact that all patients had *TBX1* deletions, only 12 had CTDs. Furthermore, the size of the deletion differed among patients. These results suggest that other genes or interactions may play a role in penetrance or the severity of the disease. Although 90% of DGS cases appear to be due to 22q11.2 deletion, other chromosomal defects with features of DiGeorge anomaly have also been described in regions 10p14 (DGS2), 4q35, 8p23 and 17p13.[19] Maybe some genes in these loci or elsewhere interact with genes in the 22q11.2 region. In our study, of 24 reported deletions, 20 (83%) were from LCR22-A to LCR22-D, two (8%) were from LCR22-A to LCR22-C and two (8%) were from LCR22-A to LCR22-B. Most 22q11 DiGeorge deletions (87%) extend from LCR22-A to LCR22-D,[4] 8% start at LCR22-A and end at LCR22-B, and 2% start at LCR22-A and end at LCR22-C. Of 20 patients with the LCR-A to LCR-D deletion, three patients had ASD/VSD and two had VSD/PFO. Another two patients with an LCR-A to LCR-D deletion had VSD/PFO. Interestingly, among our patients with ASD/VSD, one had an LCR-A to LCR-C deletion with an *HIC2* gene deletion. The *HIC2* gene is the first gene in order of physical location and there are other more distal genes in the LCR22-D region. Consequently, no direct correlation compatible with previous investigations was found between the size of the deletion and the type of CTD in our patients. Further investigation of the deletions in a larger sample of CTD patients is necessary to clarify this issue. While there is no evidence as yet that a *TBX1* mutation alone can cause DGS, mouse studies provide compelling data that *TBX1* mutations can cause severe and gene-dosage-dependent pharyngeal arch and pouch abnormalities that are strikingly similar to the DGS phenotype.[20] Tbx1 protein is a transcriptional activator, and loss of this activity has been linked to alterations in the expression of various genes involved in cardiovascular morphogenesis. Interestingly, we observed that all of the patients with 22q11 deletion also had *TBX1* gene deletion. Therefore, due to the high prevalence of *TBX1* deletion among patients with DGS in our study, it is suggested that investigation of the *TBX1* gene rather than the commonly deleted 3 Mb region could be performed in patients affected by VCFS. This would be a more economical and less time-consuming screening test in our population.

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Contributors HG and NN formulated and managed the activity plan. HA, LS and MH were involved in clinical management and patient selection. MSedghi, NN, ML and MSalehi carried out the molecular test, and checked and interpreted the results. NN, LS and BV prepared the manuscript and responded to the queries of reviewers. All authors read and approved the final manuscript.

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