European Journal of Human Genetics (2003) 11, 349–351

www.nature.com/ejhg

SHORT REPORT

DiGeorge subtypes of nonsyndromic conotruncal defects: evidence against a major role of *TBX1* Gene

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The role of the 22q11 region genes, and among them *TBX1*, in nonsyndromic conotruncal defects (CTDs) is still unclear. Mice hemizygous at the *Tbx1* locus show a remarkable incidence of heart outflow tract anomalies, of the same type commonly found in DiGeorge/Velo-cardio-facial syndrome (DGS/VCFS). Mutation analysis of the *TBX1* gene in isolated, nonsyndromic CTDs has not demonstrated any functional pathogenetic variation so far. We screened the *TBX1* gene in 41 patients affected by nonsyndromic CTDs of the DGS/VCFS subtype, principally 'atypical' tetralogy of Fallot. Besides a few polymorphisms, we did not find any pathogenetic variation. These results do not support a major role of the *TBX1* gene as responsible for human nonsyndromic CTDs.
European Journal of Human Genetics (2003) **11,** 349–351. doi:10.1038/sj.ejhg.5200956

Keywords: TBX1; conotruncal heart defects; DiGeorge/Velo-cardio-facial syndrome

Introduction

Conotruncal defects (CTDs) represent an important class of congenital heart diseases. Although they are mostly considered as complex, multifactorial disorders,[1] a number of familial cases suggesting Mendelian inheritance have been described.[2,3] CTDs, primarily interrupted aortic arch, persistent truncus arteriosus and tetralogy of Fallot (ToF), often occur in DiGeorge/Velo-cardio-facial syndrome (DGS/VCFS) patients with chromosome 22q11.2 deletion (del22q).[4] However, del22q is very rare in isolated CTDs. In a cohort of 628 CTD patients we found only a single case of del22q.[5] Even if recent studies have reported nonsyndromic ToF with *NKX2.5* and *JAG1* gene mutations,[3,6] single-gene defects are likely to account for a very minority of CTD cases. In addition, the exclusion of del22q does not modify the recurrence risk in isolated non-syndromic CTDs.[7] All the evidences, therefore, agree with the multifactorial model of transmission for isolated CTDs and suggest that many genes and pathogenetic mechanisms may be at work.

The *TBX1* gene maps to the commonly deleted DGS/VCFS region on chromosome 22q11.2 and codes for a transcription factor belonging to the T-box family,[8] probably required for growth and septation of the conotruncus.[9] Mice hemizygous at the *Tbx1* locus show a remarkable incidence of heart outflow tract anomalies, of the same type found in DGS/VCFS patients,[10,11] suggesting a role of *TBX1* in the pathogenesis of human CTDs.[10–12] However, a few independent *TBX1* gene mutation screenings in DGS/VCFS patients without del22q turn out negative.[8,10] Gong *et al*[13] have investigated *TBX1* both in nondeleted patients with features of DGS/VCFS and in a series of subjects with isolated CTDs, including aortic arch anomalies, which can occur in DGS/VCFS individuals. They found a few rare *TBX1* variants in four DGS/VCFS patients and in three isolated CTDs, but a functional role

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Received 6 August 2002; revised 13 December 2002; accepted 17 December 2002

for these variants was not demonstrated. In order to better address the issue of the link between *TBX1* and isolated CTDs, we screened *TBX1* in a large sample of patients, including some peculiar anatomical CTD subtypes which were not represented in the mutation screening previously performed.[13]

Materials and methods

A total of 41 Italian patients, ranging in age from birth to 16.5 years (mean age $\pm$SD = 3.2 $\pm$ 1.7 years), were selected on strict phenotypic and cardiac criteria. Diagnoses were obtained by echocardiography and cardiac catheterization. In addition to interrupted aortic arch type B,[14] other CTD subtypes typically associated with del22q were included in the study, such as 'atypical' ToF with absent pulmonary valve or pulmonary atresia and major aorto-pulmonary collateral arteries,[15,16] pulmonary atresia with ventricular septal defects,[17] and truncus arteriosus with truncal valve dysplasia[18] (Table 1). Patients showed neither major or minor extracardiac features of DGS/VCFS, nor deletion of 22q11 and 10p13 DiGeorge syndrome regions by standard molecular analysis.[19] Parental informed consent was obtained. The *TBX1* gene coding regions including exon–intron boundaries and 500 bp upstream the start codon were PCR amplified from genomic DNA. Primers were designed following the three splicing variant cDNA sequences and the corresponding genomic regions available on the GenBank database (AF012130, AF012131 and AF373867 and AC000091). The first eight exons are present in all the three differently spliced forms, while exon 9 and 10 primers had to be designed for each alternative cDNA (TBX1A, TBX1B and TBX1C) (Figure 1). PCR products were screened by single-strand conformation polymorphism analysis (SSCP; Genephor Unit; Amersham Pharmacia Biotech, Uppsala, Sweden). All fragments showing anomalous mobility shifts were sequenced (ABI PRISM 310 Genetic Analyser automated sequencer; Applied Biosystems, Foster City, CA, USA).

Table 1 Cardiac phenotype of the 41 screened patients

Congenital heart defects	*No. of patients*
Tetralogy of Fallot	
with pulmonary atresia and major aorto-pulmonary collateral arteries	20
with absent pulmonary valve	4
with aorto-pulmonary window	2
Truncus arteriosus with truncal valve dysplasia	11
Interrupted aortic arch type B	4
Total	41

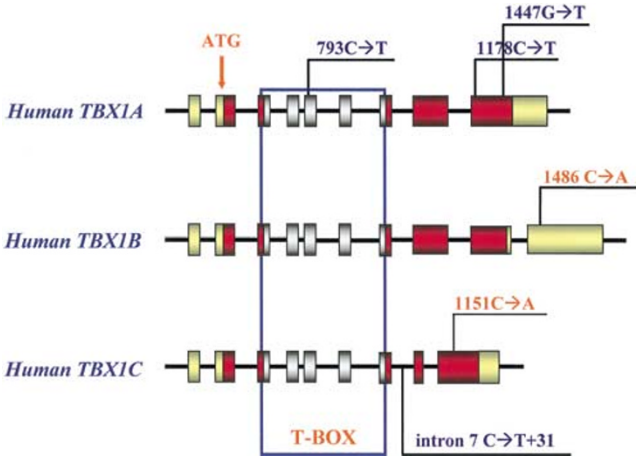

Figure 1 Genomic representation of the three alternative isoforms of the *TBX1* gene (TBX1A, TBX1B and TBX1C) and position of the detected polymorphisms. Red boxes indicate coding exons and are drawn to scale, gray boxes, pointed out by the blue line, indicate T-box coding exons, shared by all three transcripts; yellow boxes represent nontranslated sequences (5′ and 3′ UTR). The intronic regions are not to scale. The start codon position is shown by the arrow. Newly detected polymorphisms are represented in red.

Results

Six heterozygous sequence variations were detected in exons 5, 7, 9A, 9C and 10 (Figure 1). The variations in exons 5 (793C→T) and 9A (1178C→T; 1447G→T), as well as in intron 7 (C→T+31) had been previously reported and represent common polymorphisms.[8,13] Analysis of exon 9 of the TBX1C mRNA form revealed a C→A substitution at nucleotide 1151 (Pro384Gln). Analysis of exon 10 of the TBX1B mRNA displayed a C→A substitution at position 1486 in the 3′ untranslated sequences. The Exon 9C variant was identified in two out of 100 nonaffected Italian controls with an allele frequency of 1%, while the exon 10B variant was present in 21 normal controls (allele frequency 10. 5%). No pathogenic mutation was identified.

Discussion

This study has analyzed the *TBX1* gene including the 5′ and 3′ untranslated regions and the putative promoter for mutations in isolated CTD subtypes characteristically occurring in DGS/VCFS. The results provide independent confirmation that *TBX1* mutations are not a significant cause of isolated subtypes of conotruncal defects.

These findings, while agreeing with most of the previous studies on DGS/VCFS patients without 22q11 deletion, apparently contrast those observed by Gong *et al*,[13] who reported rare *TBX1* variants in isolated aortic arch anomalies. All the *TBX1* variants involved a newly discovered exon 9C, which was not screened in the previous negative studies. As remarked by Gong *et al*[13] in

their report, *TBX1* mutations may be extremely rare, accounting only for a few CTD cases. Together with a quite different distribution of the CTD subtypes in our sample, it may explain the diverging results between the two studies.

The *TBX1* gene variations so far reported on isolated CTDs are either polymorphisms, or changes with doubtful functional value.[20] We cannot anyway rule out the possibility that some of these variants, even with wide distribution in the population, are somehow involved in the pathogenesis of the patients' heart malformations, interacting with other genetic and nongenetic factors, not strictly associated with the DGS/VCFS region, in accordance with the multifactorial model. Future functional studies will address this issue.

In conclusion, the present study does not support a major role of the *TBX1* gene in the pathogenesis of human nonsyndromic CTDs. *TBX1* sequence variations are not likely to represent causative factors of this type of human cardiac defects.

Acknowledgements

This study was supported in part by grants from the Italian Ministry of Health (RF 2002) and from the Italian Ministry of Instruction, University and Research (Grant 40% to BD).

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