

A novel TBX5 loss-of-function mutation associated with sporadic dilated cardiomyopathy

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Abstract. Dilated cardiomyopathy (DCM) represents the most prevalent form of primary cardiomyopathy, and is the most common reason for heart transplantation and a major cause of congestive heart failure. Aggregating evidence demonstrates that genetic defects are associated with DCM, and a great number of mutations in >50 genes have been linked to DCM. However, DCM is a genetically heterogeneous disorder and the genetic components underpinning DCM in a significant proportion of patients remain unknown. In the present study, the coding exons and flanking exon-intron boundaries of the T-Box 5 (*TBX5*) gene, which encodes a T-box transcription factor required for normal cardiac development, were sequenced in 146 unrelated patients with sporadic DCM. The functional characteristics of the mutant *TBX5* were assayed in contrast to its wild-type counterpart by using a dual-luciferase reporter assay system. As a result, a novel heterozygous *TBX5* mutation, p.A143T, was identified in a patient with sporadic DCM. The missense mutation, which was absent in 400 control chromosomes, altered the amino acid that was completely conserved evolutionarily among species. Biological analyses revealed that the A143T mutation of *TBX5* was associated with significantly decreased transcriptional activity on the promoter of the target gene atrial natriuretic factor (*ANF*), when compared to its wild-type counterpart. Furthermore, the A143T mutation abolished the synergistic activation of the *ANF* promoter between *TBX5* and GATA binding protein 4 (*GATA4*), another crucial

transcriptional factor for heart development. To the best of our knowledge, this is the first report on the association of a *TBX5* loss-of-function mutation with an enhanced susceptibility to sporadic DCM, providing novel insight into the molecular mechanisms of the pathogenesis of DCM and suggesting potential implications for the prenatal prophylaxis and personalized treatment of this commonest primary myocardial disease.

Introduction

Dilated cardiomyopathy (DCM), clinically characterized by ventricular chamber enlargement and an impaired contraction of the left ventricle or both ventricles, represents the most prevalent type of heart muscle disease, with an estimated prevalence of 1 in 250 individuals (1). It is the most common indication for heart transplantation and a major cause of chronic congestive heart failure and sudden cardiac death (2-4). DCM has diverse etiologies with both genetic defects and environmental risk factors implicated in the pathogenesis of DCM (1-3,5). In approximately half of the cases, DCM occurs in the absence of an identifiable cause, such as coronary artery disease, viral myocarditis, cardiac valve disease, hypertension, toxic exposure, alcohol abuse, nutritional deficiency, autoimmune abnormality or metabolic disorder, and this type of DCM is defined as idiopathic or primary DCM. However, in 25-50% of cases, the familial transmission of idiopathic DCM is observed in an autosomal dominant, recessive, X-linked, or mitochondrial pattern with variable expressivity and penetrance, hence termed familial DCM, which is in contrast to sporadic DCM which is not associated with a family history (5,6). Aggregating evidence has indicated that genetic pathogenic factors play a crucial role in the pathogenesis of idiopathic DCM, and genetic mutations in >50 genes have been causally linked to idiopathic or familial DCM (1-3,7-12). Among these established DCM-associated genes, the majority encode sarcomeric, cytoskeletal, nucleoskeletal and calcium-handling proteins (2). Nevertheless, DCM is of remarkable genetic heterogeneity and the genetic basis underlying DCM in a significant proportion of patients remains unclear.

Previous studies have underlined the pivotal roles of several core cardiac transcription factors in cardiac development and structural remodeling, including the GATA zinc finger-containing transcription factor, NK homeodomain tran-

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scription factor and T-box transcription factor families (13-17), and mutations in GATA binding protein (GATA)4, GATA5, GATA6, NK2 homeobox (NKX2)-5, NKX2-6 and T-Box 5 (TBX5) have been associated with various congenital heart diseases and arrhythmias, including cardiac septal defect, tetralogy of Fallot, double outlets of the right ventricle and atrial fibrillation (18-49). Recently, mutations in GATA4, GATA5, GATA6, NKX2-5 and TBX5 have been associated with familial DCM (50-56). Additionally, mutated GATA4 has also been shown to be involved in sporadic DCM (57). However, the prevalence and spectrum of TBX5 mutations in patients with sporadic DCM remain to be investigated.

Subjects and methods

Study subjects. A cohort of 146 unrelated patients with sporadic DCM was recruited from the Han Chinese population. The controls consisted of 200 unrelated healthy individuals, who were matched to the patients with DCM in ethnicity and gender. All study participants underwent a comprehensive clinical evaluation, including a report of individual and familial histories, medical records, physical examination, two-dimensional transthoracic echocardiography with color flow Doppler, X-ray, standard 12-lead electrocardiogram and exercise tolerance testing. Cardiac catheterization, angiography, or cardiac magnetic resonance imaging was performed only if there was a strong clinical indication. The diagnosis of idiopathic DCM was made as previously described: a left ventricular end-diastolic diameter of >27 mm/m² and an ejection fraction of $<40\%$ or fractional shortening $<25\%$ in the absence of abnormal loading conditions, coronary artery disease, congenital heart lesions and other systemic diseases (50-53,58,59). The patients with co-existent conditions that may result in ventricular systolic dysfunction, such as coronary heart disease, hypertensive heart disease, valvular heart disease and viral myocarditis, were excluded from the study. The clinical studies were performed by investigators blinded to the genotypic results. This study was performed in conformity with the principles of the Declaration of Helsinki. The study protocol was reviewed and approved by the Ethics Committee of Shanghai Eighth People's Hospital, Shanghai, China. Written informed consent was obtained from all participants prior to the enrollment in the study.

Mutational screening of TBX5. A peripheral venous blood sample was prepared from each study participant, and the genomic DNA was extracted using a Wizard Genomic DNA Purification kit (Promega Corp., Madison, WI, USA). The coding exons and flanking introns of the *TBX5* gene were sequenced in the 146 unrelated patients with sporadic DCM. A total of 200 unrelated healthy control individuals were also genotyped for *TBX5*. The primer pairs used to amplify the coding regions and splicing junctions of *TBX5* by polymerase chain reaction (PCR) were designed as previously described (56). PCR was carried out using HotStar Taq DNA Polymerase (Qiagen, Hilden, Germany) on a Veriti Thermal Cycler (Applied Biosystems, Foster, CA, USA). Both strands of each PCR product were sequenced using a BigDye[®] Terminator v3.1 Cycle Sequencing kit under an ABI PRISM 3130xl DNA Analyzer (both from Applied Biosystems). The identified *TBX5* variant was validated by re-sequencing an independent PCR-generated

amplicon from the same individual, and was queried in the single nucleotide polymorphism (SNP) database at the National Center for Biotechnology Information (NCBI; <http://www.ncbi.nlm.nih.gov/>), the human gene mutation database (HGMD; <http://www.hgmd.org/>) and the 1000 Genomes Project (1000 GP) database (<http://www.1000genomes.org/>) to confirm its novelty.

Alignment of multiple TBX5 protein sequences across species. The conservation of an affected amino acid was shown by using the online MUSCLE program on the NCBI website (http://www.ncbi.nlm.nih.gov/homologene?cmd=Retrieve&dopt=MultipleAlignment&list_uids=160).

Analysis of TBX5 variation in silico. The pathogenic potential of a novel *TBX5* sequence variation was predicted by MutationTaster (an online program at: <http://www.mutation-taster.org>), which automatically yielded a probability for the variation to be either a deleterious mutation or a benign polymorphism, and PolyPhen-2 (an online program at <http://genetics.bwh.harvard.edu/pph2/>).

Plasmids and site-targeted mutagenesis. The recombinant expression plasmid, *TBX5*-pcDNA3.1, was constructed as previously described (56). The identified mutation was introduced into the wild-type *TBX5*-pcDNA3.1 construct by PCR-based site-directed mutagenesis using a QuikChange II XL Site-Directed Mutagenesis kit (Stratagene, La Jolla, CA, USA) and confirmed by sequencing. The expression vector, *GATA4*-pSSRa, and the (atrial natriuretic factor) *ANF*-luciferase (*ANF*-luc) reporter, which contains the 2600-bp 50-flanking region of the *ANF* gene and expresses Firefly luciferase, were kindly provided by Dr Ichiro Shiojima from Chiba University School of Medicine (Chiba, Japan).

Biological analysis of mutated TBX5. COS-7 cells (a fibroblast-like cell line derived from monkey kidney tissue) from our cell bank were seeded in 24-well plates and cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. Cells at approximately 90% confluency were transfected using Lipofectamine[™] 2000 transfection reagent (Invitrogen Life Technologies, Carlsbad, CA, USA). The internal control reporter plasmid pGL4.75 (hRluc/CMV; Promega Corp.), which expresses the *Renilla* luciferase, were used in transient transfection assays to normalize transfection efficiency. For each transfection, the COS-7 cells were transfected with 0.4 μ g of wild-type or mutant *TBX5*-pcDNA3.1, 1.0 μ g of *ANF*-luc reporter construct and 0.04 μ g of pGL4.75. For co-transfection experiment, 0.2 μ g of wild-type *TBX5*-pcDNA3.1 and 0.2 μ g of mutant *TBX5*-pcDNA3.1 were used in the presence of 0.4 μ g of *ANF*-luc and 0.04 μ g of pGL4.75. For the synergistic activation experiment, 0.4 μ g of wild-type *GATA4*-pSSRa was added. Firefly and *Renilla* luciferase activities were measured with the Dual-Glo Luciferase Assay system (Promega Corp.) 48 h after transfection. The activity of the *ANF* promoter was expressed as the fold activation of Firefly luciferase relative to *Renilla* luciferase. Three independent experiments were conducted at minimum for each transfection.

Statistical analysis. Continuous variables are presented as the means \pm SD and the Student's unpaired t-test was used

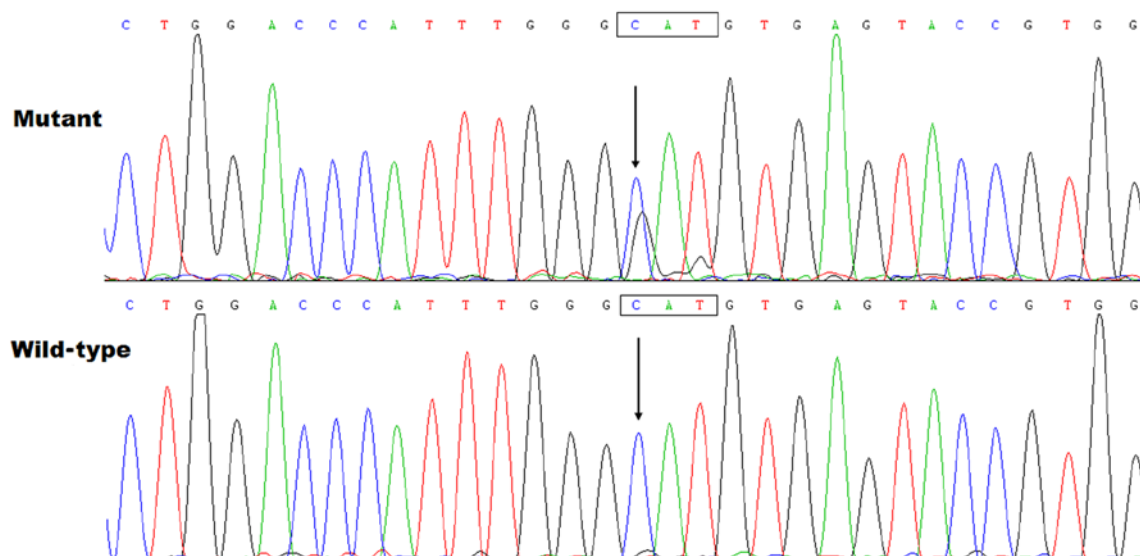


Figure 1. Sequence electropherograms showing the TBX5 mutation and its corresponding control. The arrows indicate the heterozygous nucleotides of G/A in the patient (mutant), or the homozygous nucleotides of G/G in the corresponding control individual (wild-type). The rectangle indicates the nucleotides comprising a codon of TBX5.

to compare the continuous variables between 2 groups. Non-continuous and categorical variables are presented as frequencies or percentages and were compared using Pearson's χ^2 test or Fisher's exact test where appropriate. All statistical analyses were two-sided and P-values <0.05 were considered to indicate a statistically significant difference.

Results

Baseline clinical characteristics of the study population. In this case-control study, 146 unrelated patients with sporadic DCM were clinically evaluated in contrast to 200 unrelated healthy control individuals. The patients presented with the typical phenotype of DCM as previously described by Elliott *et al* (58). The control individuals had normal echocardiographic parameters without any evidence of heart disease. All the study participants stated to have no family history of DCM, and none of them had known risk factors or comorbidities for DCM, such as coronary artery disease, hypertension, viral myocarditis, valvular heart disease, congenital heart disease, drug abuse or exposure to toxicants. The baseline clinical characteristics of the study subjects are summarized in Table I.

Identification of a novel TBX5 mutation. By sequence analysis of TBX5, a heterozygous variation was identified in 1 of the 146 unrelated patients with sporadic DCM, with a mutational prevalence of approximately 0.68%. Specifically, a transversion of guanine into adenine at nucleotide position 143 (c.427G>A), predicting the substitution of threonine for alanine at amino acid 143 (p.A143T), was identified in a 47-year-old female with DCM. She stated to have not positive family history of DCM, and had no overt congenital cardiovascular abnormalities or forelimb malformations. The sequence electropherograms showing the detected heterozygous TBX5 variation in contrast to its corresponding control sequence are presented in Fig. 1. The schematic diagram of TBX5 protein showing the T-box structural domain and the location of the mutation identified

Table I. Baseline clinical characteristics of the study subjects and control individuals.

Variables	Patients (n=146)	Controls (n=200)	P-value
Age (years)	54±10	55±9	0.3309
Male gender (%)	72 (49)	98 (49)	0.9538
SBP (mmHg)	119±13	126±12	<0.0001
DBP (mmHg)	80±8	83±7	0.0002
HR (bpm)	87±14	75±12	<0.0001
LVEDD (mm)	69±7	46±6	<0.0001
LVESD (mm)	59±7	36±6	<0.0001
LVEF (%)	38±8	63±7	<0.0001
LVFS (%)	19±6	31±5	<0.0001
NYHA function class (%)			
I	17 (12)	NA	NA
II	45 (31)	NA	NA
III	62 (43)	NA	NA
IV	21 (14)	NA	NA

SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LVEF, left ventricular ejection fraction; LVFS, left ventricular fractional shortening; NYHA, New York Heart Association; NA, not applicable or not available.

in this study is illustrated in Fig. 2. The mutation was not observed in the 400 control chromosomes and it was not found in the SNP, HGM or 1000 GP databases, which were consulted again on March 19, 2015, suggesting a novel mutation.

Alignment of multiple TBX5 protein sequences across species. As shown in Fig. 3, a cross-species alignment of multiple TBX5 protein sequences displayed that the alanine at amino

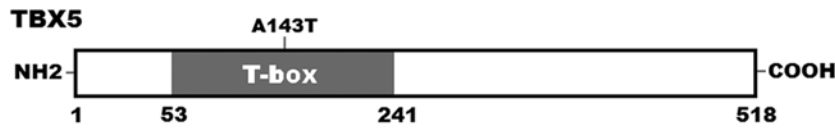


Figure 2. Schematic diagram of human TBX5 protein structure with the dilated cardiomyopathy (DCM)-related mutation shown. The mutation identified in a patient with sporadic DCM shown above the structural domain. NH2, amino-terminus; T-box, T-box domain; COOH, carboxyl-terminus.

		118	A143T ↓	168
NP_000183.2	(Human)	--- DNKWSVTGKAEPAMPGRLYVHPDSP	A	TGAHWMRQLVSFQKLKLTNNHLDPF ---
XP_001154140.2	(Chimpanzee)	--- DNKWSVTGKAEPAMPGRLYVHPDSP	A	TGAHWMRQLVSFQKLKLTNNHLDPF ---
XP_001111737.1	(Monkey)	--- DNKWSVTGKAEPAMPGRLYVHPDSP	A	TGAHWMRQLVSFQKLKLTNNHLDPF ---
XP_005636327.1	(Dog)	--- DNKWSVTGKAEPAMPGRLYVHPDSP	A	TGAHWMRQLVSFQKLKLTNNHLDPF ---
NP_001179678.1	(Cattle)	--- DNKWSVTGKAEPAMPGRLYVHPDSP	A	TGAHWMRQLVSFQKLKLTNNHLDPF ---
NP_035667.1	(Mouse)	--- DNKWSVTGKAEPAMPGRLYVHPDSP	A	TGAHWMRQLVSFQKLKLTNNHLDPF ---
NP_001009964.1	(Rat)	--- DNKWSVTGKAEPAMPGRLYVHPDSP	A	TGAHWMRQLVSFQKLKLTNNHLDPF ---
NP_989504.1	(Fowl)	--- DNKWSVTGKAEPAMPGRLYVHPDSP	A	TGAHWMRQLVSFQKLKLTNNHLDPF ---
NP_570990.1	(Zebrafish)	--- DNKWSVTGKAEPAMPGRLYVHPDSP	A	TGAHWMRQLVSFQKLKLTNNHLDPF ---
NP_001185697.1	(Frog)	--- DNKWSVTGKAEPAMPGRLYVHPDSP	A	TGAHWMRQLVSFQKLKLTNNHLDPF ---

Figure 3. Alignment of multiple TBX5 protein sequences among species. The altered amino acid of A143 in TBX5 is completely conserved evolutionarily among various species.

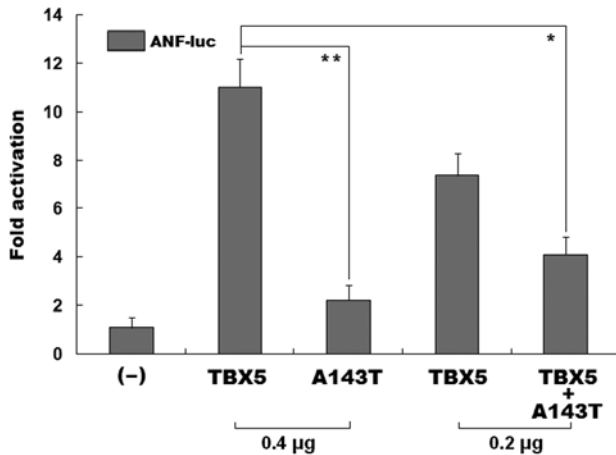


Figure 4. Reduced transcriptional functional associated with TBX5 mutation. Activation of atrial natriuretic factor (ANF) promoter driven luciferase in COS-7 cells by wild-type TBX5 or the TBX5 mutant, A143T, alone or in combination, showed significantly reduced transcriptional activity by mutant protein. Experiments were performed in triplicate, and the mean \pm standard deviations are shown. ** $P=0.0003$ ($t=11.6223$), * $P=0.0010$ ($t=8.6439$), respectively, when compared with wild-type TBX5. ANF-luc, ANF-luciferase.

acid position 143 of human TBX5 was completely conserved evolutionarily among species, implying that the amino acid is functionally important.

Causative potential of a TBX5 variation. The TBX5 sequence variation of c.427G>A was predicted to be pathogenic by MutationTaster, with a P-value of 0.9999999932. No SNPs in the altered region were reported in the MutationTaster database. The amino acid substitution of p.A143T was also predicted to be possibly damaging by another online program, PolyPhen-2,

with a score of 0.994 (sensitivity, 0.46; specificity, 0.96). These results indicate that the TBX5 mutation contributes to DCM in this mutation carrier.

Reduced transcriptional activity of the mutant TBX5. As shown in Fig. 4, the same amount (0.4 μ g) of wild-type TBX5 and its mutant counterpart, A143T, activated the ANF promoter by approximately 11- and 2-fold, respectively. When wild-type TBX5 was co-expressed with the same amount (0.2 μ g) of the TBX5 mutant, A143T, the induced activation of the ANF promoter was approximately 4-fold; while 0.2 μ g of wild-type TBX5 alone activated the ANF promoter by approximately 7-fold. These functional data reveal that the TBX5 mutant, A143T, had a significantly reduced transcriptional activity and a dominant-negative effect on its wild-type counterpart.

Diminished synergistic transcriptional activity of the TBX5 mutant. As shown in Fig. 5, in the presence of 0.4 μ g of GATA4, the same amount (0.4 μ g) of wild-type TBX5 and the TBX5 mutant, A143T, activated the ANF promoter by approximately 25- and 7-fold, respectively. When wild-type TBX5 was co-expressed with the same amount (0.2 μ g) of the TBX5 mutant, A143T, the induced activation of the ANF promoter was approximately 14-fold. These functional data reveal that the TBX5 mutant, A143T, had a significantly diminished synergistic transcriptional activity with GATA4.

Discussion

In this study, a novel heterozygous mutation in TBX5, p.A143T, was identified in a patient with sporadic DCM. The mutation was absent in the 400 referral chromosomes, and altered the amino

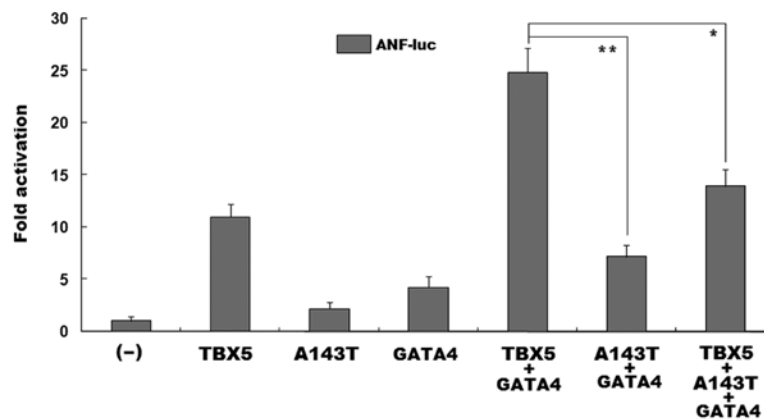


Figure 5. Diminished synergistic transcriptional activity between mutant TBX5 and GATA4. In the presence of GATA4, the activation of the atrial natriuretic factor (*ANF*) promoter-driven luciferase in COS-7 cells by wild-type TBX5 or A143T-mutant TBX5 (A143T), alone or in combination, showed significantly diminished transcriptional activity by mutant protein. Experiments were performed in triplicate, and the mean \pm standard deviations are shown. ** $P=0.0003$ ($t=12.1366$), * $P=0.0025$ ($t=6.7513$), respectively, when compared with wild-type TBX5. ANF-luc, ANF-luciferase.

acid that was completely conserved evolutionarily. Functional analyses revealed that the TBX5 mutant, A143T, was associated with a significantly decreased transcriptional activity and had a dominant-negative effect on its wild-type counterpart. Furthermore, the mutation diminished the synergistic activation between TBX5 and GATA4. Therefore, it is likely that mutant TBX5 predisposes the mutation carrier to DCM. To the best of our knowledge, this is the first clinical study that demonstrates the association of TBX5 loss-of-function mutation with an enhanced susceptibility to sporadic DCM.

To date, 17 members of the T-box containing transcription factor family have been discovered in mammals, of which 6 members, including TBX1, TBX18 and TBX20 of the TBX1 subfamily, and TBX2, TBX3 and TBX5 of the TBX2 subfamily, are expressed in the heart (16). In humans, the *TBX5* gene, which maps to chromosome 12q24.1 and contains 8 coding exons, encodes a protein of 518 amino acids. The TBX5 protein has an important structural domain termed T-box, which is highly conserved throughout members of the T-box family and across various species. The T-box motif is essential for the specific binding to target DNA and the protein-protein interactions, and previous studies have substantiated that TBX5 regulates the expression of multiple important target genes expressed in the heart during embryogenesis, including *ANF*, *CX40* and serum response factor (*SRF*), alone or in synergy with transcriptionally cooperative partners, such as GATA4 and NKX2-5 (16,18,38,60,61). In the present study, the TBX5 mutation identified in a patient with sporadic DCM is located in the T-box domain, and functional analyses unveiled that the mutation led to a significantly reduced transcriptional activity of TBX5, alone or synergistically with GATA4, and exerted a dominant-negative effect on wild-type TBX5. These findings suggest that haploinsufficiency or a dominant-negative effect resulted from the TBX5 mutation may be an alternative pathological mechanism of the pathogenesis of sporadic DCM.

The association of *TBX5* loss-of-function mutation with an increased vulnerability to familial DCM has been reported previously. Zhang *et al* (56) sequenced the coding regions and splice junction sites of *TBX5* in a cohort of 190 unrelated patients with idiopathic DCM, and found a novel heterozygous mutation (p.S154A) in an index patient with a positive family

history, with a mutational prevalence of approximately 0.53%. Genetic analysis of the proband's pedigree revealed that the mutation co-segregated with DCM transmitted in an autosomal dominant pattern with complete penetrance. Biological assays revealed that the mutation significantly decreased the transcription activating function of TBX5, as well as its synergism with partners GATA4 and NKX2-5 at the *ANF* promoter. Similarly, in the present study, a novel TBX5 loss-of-function mutation, p.A143T, was linked to sporadic DCM. These observational results suggest that genetically compromised TBX5 contributes to the pathogenesis of DCM in a subset of patients.

The discovery that functionally impaired TBX5 predisposes to DCM may be partially attributed to the abnormal development and structural remodeling of the heart. In vertebrates and humans, TBX5 is amply expressed in the embryonic and adult hearts, and is required for normal cardiac development and structural remodeling, including cellular specification, proliferation, differentiation and migration, tissue patterning and morphogenesis (62). In mice, TBX5 is highly expressed in the cardiac crescent, linear heart tube, common atrium, left ventricle, left-side ventricular septum, trabeculae of the right ventricle, and the atrial aspect of the atrioventricular valves (63). Mice with a homozygous deletion of two *Tbx5* alleles died by E10.5, due to failure of cardiac looping, hypoplasia of sinuatria and left ventricle; while mice with a heterozygous deletion of one *Tbx5* allele presented with atrial septal defects, ventricular septal defects, endocardial cushion defects, hypoplastic left heart, aberrant trabeculation, and atrioventricular block, similar with what were observed in patients with Holt-Oram syndrome (64). Moreover, in mice TBX5 and GATA4 are co-expressed and interact physically in the developing atria and ventricles, and mice with doubly heterozygous deletion of *Tbx5* and *Gata4* suffered embryonic lethality, thin atrial and ventricular myocardium with reduced cell proliferation and atrioventricular septation defects (65). Taken together, these results from experimental animals support that TBX5 loss-of-function mutation is involved in DCM in humans.

It has been substantiated that TBX5 physically interacts with multiple core cardiac transcriptional factors, including GATA4, NKX2-5, MEF2c and TBX20, to form a transcriptional complex resulting in the synergistic activation of target

genes required for proper cardiac development, including *ANF*, *CX40*, *SRF*, *MHY6* and *ID2* (16), and loss-of-function mutations in several transcriptional cooperative partners of *TBX5*, including *NKX2-5*, *GATA4*, *GATA5*, *GATA6* and *TBX20*, have been reported to be responsible for DCM in humans (50-57,66). Therefore, genetically defective *TBX5* may contribute to DCM by decreasing the expression of some target genes important for cardiac development and structural remodeling.

Notably, *TBX5* mutations have previously been related to Holt-Oram syndrome, an autosomal dominant disease characteristic of congenital cardiac defects and anterior upper limb deformities. Cardiac phenotypes constitute a wide spectrum of cardiovascular developmental anomalies, including atrial septal defect, ventricular septal defect, tetralogy of Fallot, atrioventricular septal defect, patent ductus arteriosus, mitral valve defect and aberrant pulmonary vein, as well as cardiac conduction block and atrial fibrillation (37,48,67). There are three variations of Holt-Oram syndrome which have been encountered in clinical practice: patients may have only cardiovascular abnormalities (4%), only forelimb malformations (27%), or both (69%), and these cardiac and skeletal deformations vary widely ranging from mild to severe, even within families (48). In the present study, in addition to DCM, the mutation carrier had no apparent cardiac or skeletal anomaly. Different genetic backgrounds, epigenetic modifiers and functional characteristics of gene mutations (loss-of-function, dominant-negative, or gain-of-function effect) as well as its temporal and spatial effects during cardiac development (germline or somatic) are potential explanations for the pronounced variability in clinical presentation (46).

In conclusion, to the best of our knowledge, this study is the first to present an association of a *TBX5* loss-of-function mutation with an increased susceptibility to sporadic DCM, providing novel insight into the molecular mechanisms of the pathogenesis DCM, and suggesting potential implications for prenatal prophylaxis and personalized treatment of the most common type of primary cardiomyopathy.

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References

- McNally EM, Golbus JR and Puckelwartz MJ: Genetic mutations and mechanisms in dilated cardiomyopathy. *J Clin Invest* 123: 19-26, 2013.
- Hershberger RE, Hedges DJ and Morales A: Dilated cardiomyopathy: the complexity of a diverse genetic architecture. *Nat Rev Cardiol* 10: 531-547, 2013.
- Garcia-Pavia P, Cobo-Marcos M, Guzzo-Merello G, Gomez-Bueno M, Bornstein B, Lara-Pezzi E, Segovia J and Alonso-Pulpon L: Genetics in dilated cardiomyopathy. *Biomarkers Med* 7: 517-533, 2013.
- Koutalas E, Kanoupakis E and Vardas P: Sudden cardiac death in non-ischemic dilated cardiomyopathy: a critical appraisal of existing and potential risk stratification tools. *Int J Cardiol* 167: 335-341, 2013.
- Yoshikawa T: Contribution of acquired factors to the pathogenesis of dilated cardiomyopathy. -The cause of dilated cardiomyopathy: genetic or acquired? (acquired-side)-. *Circ J* 75: 1766-1773, 2011.
- Kamisago M, Sharma SD, DePalma SR, Solomon S, Sharma P, McDonough B, Smoot L, Mullen MP, Woolf PK, Wigle ED, *et al*: Mutations in sarcomere protein genes as a cause of dilated cardiomyopathy. *N Engl J Med* 343: 1688-1696, 2000.
- Arndt AK, Schafer S, Drenckhahn JD, Sabeh MK, Plovie ER, Caliebe A, Klopocki E, Musso G, Werdich AA, Kalwa H, *et al*: Fine mapping of the 1p36 deletion syndrome identifies mutation of *PRDM16* as a cause of cardiomyopathy. *Am J Hum Genet* 93: 67-77, 2013.
- Pankuweit S, Ruppert V, Jónsdóttir T, Müller HH and Meyer T: German Competence Network of Heart Failure: The HLA class II allele *DQB1 0309* is associated with dilated cardiomyopathy. *Gene* 531: 180-183, 2013.
- Wahbi K, Béhin A, Bécane HM, Leturcq F, Cossée M, Laforêt P, Stojkovic T, Carlier P, Toussaint M, Gaxotte V, *et al*: Dilated cardiomyopathy in patients with mutations in *anoctamin 5*. *Int J Cardiol* 168: 76-79, 2013.
- Ruppert V, Meyer T, Richter A, Maisch B and Pankuweit S: German Competence Network of Heart Failure: Identification of a missense mutation in the melusin-encoding *ITGB1BP2* gene in a patient with dilated cardiomyopathy. *Gene* 512: 206-210, 2013.
- Agrawal PB, Pierson CR, Joshi M, Liu X, Ravenscroft G, Moghadaszadeh B, Talabere T, Viola M, Swanson LC, Halilović G, *et al*: *SPEG* interacts with myotubularin, and its deficiency causes centronuclear myopathy with dilated cardiomyopathy. *Am J Hum Genet* 95: 218-226, 2014.
- Matsa LS, Sagurthi SR, Ananthapur V, Nalla S and Nallari P: Endothelin 1 gene as a modifier in dilated cardiomyopathy. *Gene* 548: 256-262, 2014.
- Pikkariainen S, Tokola H, Kerkelä R and Ruskoaho H: GATA transcription factors in the developing and adult heart. *Cardiovasc Res* 63: 196-207, 2004.
- Cai H, Katoh-Kurasawa M, Muramoto T, Santhanam B, Long Y, Li L, Ueda M, Iglesias PA, Shaulsky G and Devreotes PN: Nucleocytoplasmic shuttling of a GATA transcription factor functions as a development timer. *Science* 343: 1249531, 2014.
- Akazawa H and Komuro I: Cardiac transcription factor *Csx/Nkx2-5*: Its role in cardiac development and diseases. *Pharmacol Ther* 107: 252-268, 2005.
- Greulich F, Rudat C and Kispert A: Mechanisms of T-box gene function in the developing heart. *Cardiovasc Res* 91: 212-222, 2011.
- Oka T, Xu J and Molkenstein JD: Re-employment of developmental transcription factors in adult heart disease. *Semin Cell Dev Biol* 18: 117-131, 2007.
- Garg V, Kathiriyia IS, Barnes R, Schluterman MK, King IN, Butler CA, Rothrock CR, Eapen RS, Hirayama-Yamada K, Joo K, *et al*: GATA4 mutations cause human congenital heart defects and reveal an interaction with *TBX5*. *Nature* 424: 443-447, 2003.
- Rajagopal SK, Ma Q, Obler D, Shen J, Manichaikul A, Tomita-Mitchell A, Boardman K, Briggs C, Garg V, Srivastava D, *et al*: Spectrum of heart disease associated with murine and human GATA4 mutation. *J Mol Cell Cardiol* 43: 677-685, 2007.
- Yang YQ, Wang J, Liu XY, Chen XZ, Zhang W and Wang XZ: Mutation spectrum of GATA4 associated with congenital atrial septal defects. *Arch Med Sci* 9: 976-983, 2013.
- Yang YQ, Gharibeh L, Li RG, Xin YF, Wang J, Liu ZM, Qiu XB, Xu YJ, Xu L, Qu XK, *et al*: GATA4 loss-of-function mutations underlie familial tetralogy of Fallot. *Hum Mutat* 34: 1662-1671, 2013.
- Xiang R, Fan LL, Huang H, Cao BB, Li XP, Peng DQ and Xia K: A novel mutation of GATA4 (K319E) is responsible for familial atrial septal defect and pulmonary valve stenosis. *Gene* 534: 320-323, 2014.
- Jiang JQ, Li RG, Wang J, Liu XY, Xu YJ, Fang WY, Chen XZ, Zhang W, Wang XZ and Yang YQ: Prevalence and spectrum of GATA5 mutations associated with congenital heart disease. *Int J Cardiol* 165: 570-573, 2013.
- Wei D, Bao H, Zhou N, Zheng GF, Liu XY and Yang YQ: GATA5 loss-of-function mutation responsible for the congenital ventriculoseptal defect. *Pediatr Cardiol* 34: 504-511, 2013.
- Wei D, Bao H, Liu XY, Zhou N, Wang Q, Li RG, Xu YJ and Yang YQ: GATA5 loss-of-function mutations underlie tetralogy of fallot. *Int J Med Sci* 10: 34-42, 2013.

26. Shi LM, Tao JW, Qiu XB, Wang J, Yuan F, Xu L, Liu H, Li RG, Xu YJ, Wang Q, *et al*: GATA5 loss-of-function mutations associated with congenital bicuspid aortic valve. *Int J Mol Med* 33: 1219-1226, 2014.
27. Huang RT, Xue S, Xu YJ, Zhou M and Yang YQ: Somatic GATA5 mutations in sporadic tetralogy of Fallot. *Int J Mol Med* 33: 1227-1235, 2014.
28. Zheng GF, Wei D, Zhao H, Zhou N, Yang YQ and Liu XY: A novel GATA6 mutation associated with congenital ventricular septal defect. *Int J Mol Med* 29: 1065-1071, 2012.
29. Huang RT, Xue S, Xu YJ and Yang YQ: Somatic mutations in the GATA6 gene underlie sporadic tetralogy of Fallot. *Int J Mol Med* 31: 51-58, 2013.
30. Schott JJ, Benson DW, Basson CT, Pease W, Silberbach GM, Moak JP, Maron BJ, Seidman CE and Seidman JG: Congenital heart disease caused by mutations in the transcription factor NKX2-5. *Science* 281: 108-111, 1998.
31. Huang W, Meng H, Qiao Y, Pang S, Chen D and Yan B: Two novel and functional DNA sequence variants within an upstream enhancer of the human NKX2-5 gene in ventricular septal defects. *Gene* 524: 152-155, 2013.
32. Qu XK, Qiu XB, Yuan F, Wang J, Zhao CM, Liu XY, Zhang XL, Li RG, Xu YJ, Hou XM, *et al*: A novel NKX2.5 loss-of-function mutation associated with congenital bicuspid aortic valve. *Am J Cardiol* 114: 1891-1895, 2014.
33. Heathcote K, Braybrook C, Abushaban L, Guy M, Khetyar ME, Patton MA, Carter ND, Scambler PJ and Syrris P: Common arterial trunk associated with a homeodomain mutation of NKX2.6. *Hum Mol Genet* 14: 585-593, 2005.
34. Ta-Shma A, El-lahham N, Edvardson S, Stepensky P, Nir A, Perles Z, Gavri S, Golender J, Yaakobi-Simhayoff N, Shaag A, *et al*: Conotruncal malformations and absent thymus due to a deleterious NKX2-6 mutation. *J Med Genet* 51: 268-270, 2014.
35. Zhao L, Ni SH, Liu XY, Wei D, Yuan F, Xu L, Xin-Li, Li RG, Qu XK, Xu YJ, *et al*: Prevalence and spectrum of Nkx2.6 mutations in patients with congenital heart disease. *Eur J Med Genet* 57: 579-586, 2014.
36. Wang J, Mao JH, Ding KK, Xu WJ, Liu XY, Qiu XB, Li RG, Qu XK, Xu YJ, Huang RT, *et al*: A novel NKX2.6 mutation associated with congenital ventricular septal defect. *Pediatr Cardiol* 36: 646-656, 2015.
37. Baban A, Postma AV, Marini M, Trocchio G, Santilli A, Pelegrini M, Sirlito P, Lerone M, Albanese SB, Barnett P, *et al*: Identification of TBX5 mutations in a series of 94 patients with Tetralogy of Fallot. *Am J Med Genet A* 164A: 3100-3107, 2014.
38. McCulley DJ and Black BL: Transcription factor pathways and congenital heart disease. *Curr Top Dev Biol* 100: 253-277, 2012.
39. Andersen TA, Troelsen KL and Larsen LA: Of mice and men: molecular genetics of congenital heart disease. *Cell Mol Life Sci* 71: 1327-1352, 2014.
40. Wang J, Sun YM and Yang YQ: Mutation spectrum of the GATA4 gene in patients with idiopathic atrial fibrillation. *Mol Biol Rep* 39: 8127-8135, 2012.
41. Wang XH, Huang CX, Wang Q, Li RG, Xu YJ, Liu X, Fang WY and Yang YQ: A novel GATA5 loss-of-function mutation underlies lone atrial fibrillation. *Int J Mol Med* 31: 43-50, 2013.
42. Li J, Liu WD, Yang ZL and Yang YQ: Novel GATA6 loss-of-function mutation responsible for familial atrial fibrillation. *Int J Mol Med* 30: 783-790, 2012.
43. Xie WH, Chang C, Xu YJ, Li RG, Qu XK, Fang WY, Liu X and Yang YQ: Prevalence and spectrum of Nkx2.5 mutations associated with idiopathic atrial fibrillation. *Clinics (Sao Paulo)* 68: 777-784, 2013.
44. Huang RT, Xue S, Xu YJ, Zhou M and Yang YQ: A novel NKX2.5 loss-of-function mutation responsible for familial atrial fibrillation. *Int J Mol Med* 31: 1119-1126, 2013.
45. Perera JL, Johnson NM, Judge DP and Crosson JE: Novel and highly lethal NKX2.5 missense mutation in a family with sudden death and ventricular arrhythmia. *Pediatr Cardiol* 35: 1206-1212, 2014.
46. Yu H, Xu JH, Song HM, Zhao L, Xu WJ, Wang J, Li RG, Xu L, Jiang WF, Qiu XB, *et al*: Mutational spectrum of the NKX2-5 gene in patients with lone atrial fibrillation. *Int J Med Sci* 11: 554-563, 2014.
47. Wang J, Zhang DF, Sun YM, Li RG, Qiu XB, Qu XK, Liu X, Fang WY and Yang YQ: NKX2-6 mutation predisposes to familial atrial fibrillation. *Int J Mol Med* 34: 1581-1590, 2014.
48. Postma AV, van de Meerakker JB, Mathijssen IB, Barnett P, Christoffels VM, Ilgun A, Lam J, Wilde AA, Lekanne Deprez RH and Moorman AF: A gain-of-function TBX5 mutation is associated with atypical Holt-Oram syndrome and paroxysmal atrial fibrillation. *Circ Res* 102: 1433-1442, 2008.
49. Hong K and Xiong Q: Genetic basis of atrial fibrillation. *Curr Opin Cardiol* 29: 220-226, 2014.
50. Li RG, Li L, Qiu XB, Yuan F, Xu L, Li X, Xu YJ, Jiang WF, Jiang JQ, Liu X, *et al*: GATA4 loss-of-function mutation underlies familial dilated cardiomyopathy. *Biochem Biophys Res Commun* 439: 591-596, 2013.
51. Zhao L, Xu JH, Xu WJ, Yu H, Wang Q, Zheng HZ, Jiang WF, Jiang JF and Yang YQ: A novel GATA4 loss-of-function mutation responsible for familial dilated cardiomyopathy. *Int J Mol Med* 33: 654-660, 2014.
52. Zhang XL, Dai N, Tang K, Chen YQ, Chen W, Wang J, Zhao CM, Yuan F, Qiu XB, Qu XK, *et al*: GATA5 loss-of-function mutation in familial dilated cardiomyopathy. *Int J Mol Med* 35: 763-770, 2015.
53. Xu L, Zhao L, Yuan F, Jiang WF, Liu H, Li RG, Xu YJ, Zhang M, Fang WY, Qu XK, *et al*: GATA6 loss-of-function mutations contribute to familial dilated cardiomyopathy. *Int J Mol Med* 34: 1315-1322, 2014.
54. Costa MW, Guo G, Wolstein O, Vale M, Castro ML, Wang L, Otway R, Riek P, Cochrane N, Furtado M, *et al*: Functional characterization of a novel mutation in NKX2-5 associated with congenital heart disease and adult-onset cardiomyopathy. *Circ Cardiovasc Genet* 6: 238-247, 2013.
55. Yuan F, Qiu XB, Li RG, Qu XK, Wang J, Xu YJ, Liu X, Fang WY, Yang YQ and Liao DN: A novel NKX2-5 loss-of-function mutation predisposes to familial dilated cardiomyopathy and arrhythmias. *Int J Mol Med* 35: 478-486, 2015.
56. Zhang XL, Qiu XB, Yuan F, Wang J, Zhao CM, Li RG, Xu L, Xu YJ, Shi HY, Hou XM, *et al*: TBX5 loss-of-function mutation contributes to familial dilated cardiomyopathy. *Biochem Biophys Res Commun* 459: 166-171, 2015.
57. Li J, Liu WD, Yang ZL, Yuan F, Xu L, Li RG and Yang YQ: Prevalence and spectrum of GATA4 mutations associated with sporadic dilated cardiomyopathy. *Gene* 548: 174-181, 2014.
58. Elliott P, O'Mahony C, Syrris P, Evans A, Rivera Sorensen C, Sheppard MN, Carr-White G, Pantazis A and McKenna WJ: Prevalence of desmosomal protein gene mutations in patients with dilated cardiomyopathy. *Circ Cardiovasc Genet* 3: 314-322, 2010.
59. Richardson P, McKenna W, Bristow M, Maisch B, Mautner B, O'Connell J, Olsen E, Thiene G, Goodwin J, Gyrfas I, *et al*: Report of the 1995 World Health Organization/International Society and Federation of Cardiology Task Force on the Definition and Classification of cardiomyopathies. *Circulation* 93: 841-842, 1996.
60. Hiroi Y, Kudoh S, Monzen K, Ikeda Y, Yazaki Y, Nagai R and Komuro I: Tbx5 associates with Nkx2-5 and synergistically promotes cardiomyocyte differentiation. *Nat Genet* 28: 276-280, 2001.
61. Stennard FA, Costa MW, Elliott DA, Rankin S, Haast SJ, Lai D, McDonald LP, Niederreither K, Dolle P, Bruneau BG, *et al*: Cardiac T-box factor Tbx20 directly interacts with Nkx2-5, GATA4, and GATA5 in regulation of gene expression in the developing heart. *Dev Biol* 262: 206-224, 2003.
62. Plageman TF Jr and Yutzy KE: T-box genes and heart development: putting the 'T' in heart. *Dev Dyn* 232: 11-20, 2005.
63. Bruneau BG, Logan M, Davis N, Levi T, Tabin CJ, Seidman JG and Seidman CE: Chamber-specific cardiac expression of Tbx5 and heart defects in Holt-Oram syndrome. *Dev Biol* 211: 100-108, 1999.
64. Bruneau BG, Nemer G, Schmitt JP, Charron F, Robitaille L, Caron S, Conner DA, Gessler M, Nemer M, Seidman CE, *et al*: A murine model of Holt-Oram syndrome defines roles of the T-box transcription factor Tbx5 in cardiogenesis and disease. *Cell* 106: 709-721, 2001.
65. Misra C, Chang SW, Basu M, Huang N and Garg V: Disruption of myocardial Gata4 and Tbx5 results in defects in cardiomyocyte proliferation and atrioventricular septation. *Hum Mol Genet* 23: 5025-5035, 2014.
66. Kirk EP, Sunde M, Costa MW, Rankin SA, Wolstein O, Castro ML, Butler TL, Hyun C, Guo G, Otway R, *et al*: Mutations in cardiac T-box factor gene TBX20 are associated with diverse cardiac pathologies, including defects of septation and valvulogenesis and cardiomyopathy. *Am J Hum Genet* 81: 280-291, 2007.
67. Böhm J, Heinritz W, Craig A, Vujic M, Ekman-Joelsson BM, Kohlhaas J and Froster U: Functional analysis of the novel TBX5 c.1333delC mutation resulting in an extended TBX5 protein. *BMC Med Genet* 9: 88, 2008.