Biochemical and Biophysical Research Communications 459 (2015) 166–171

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

TBX5 loss-of-function mutation contributes to familial dilated cardiomyopathy

Xian-Ling Zhang [a], Xing-Biao Qiu [b], Fang Yuan [b], Juan Wang [c], Cui-Mei Zhao [c], Ruo-Gu Li [b], Lei Xu [b], Ying-Jia Xu [b], Hong-Yu Shi [b], Xu-Min Hou [b], Xin-Kai Qu [b], Ya-Wei Xu [a, **], Yi-Qing Yang [b, d, e, *]

[a] Department of Cardiology, Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai 200072, China
[b] Department of Cardiology, Shanghai Chest Hospital, Shanghai Jiao Tong University, Shanghai 200030, China
[c] Department of Cardiology, Tongji Hospital, Tongji University School of Medicine, Shanghai 200065, China
[d] Department of Cardiovascular Research Laboratory, Shanghai Chest Hospital, Shanghai Jiao Tong University, Shanghai 200030, China
[e] Department of Central Laboratory, Shanghai Chest Hospital, Shanghai Jiao Tong University, Shanghai 200030, China

ARTICLE INFO

Article history:
Received 7 February 2015
Available online 26 February 2015

Keywords:
Dilated cardiomyopathy
Genetics
Transcription factor
TBX5
Reporter gene assay

ABSTRACT

The cardiac T-box transcription factor TBX5 is crucial for proper cardiovascular development, and mutations in TBX5 have been associated with various congenital heart diseases and arrhythmias in humans. However, whether mutated TBX5 contributes to dilated cardiomyopathy (DCM) remains unclear. In this study, the coding exons and flanking introns of the *TBX5* gene were sequenced in 190 unrelated patients with idiopathic DCM. The available family members of the index patient carrying an identified mutation and 200 unrelated ethnically matched healthy individuals used as controls were genotyped for *TBX5*. The functional characteristics of the mutant TBX5 were explored in contrast to its wild-type counterpart by using a dual-luciferase reporter assay system. As a result, a novel heterozygous TBX5 mutation, p.S154A, was identified in a family with DCM inherited in an autosomal dominant pattern, which co-segregated with DCM in the family with complete penetrance. The missense mutation was absent in 400 control chromosomes and the altered amino acid was completely conserved evolutionarily across various species. Functional assays revealed that the mutant TBX5 had significantly decreased transcriptional activity. Furthermore, the mutation markedly diminished the synergistic activation of TBX5 with NKX2-5 or GATA4, other two transcription factors causatively linked to DCM. This study firstly associates TBX5 loss-of-function mutation with enhanced susceptibility to DCM, providing novel insight into the molecular mechanisms of DCM, and suggesting the potential implications in the development of new treatment strategies for this common form of myocardial disorder.

1. Introduction

Dilated cardiomyopathy (DCM), characterized by progressive ventricular dilatation and systolic dysfunction in the absence of associated conditions such as hypertension, valvular heart disease and coronary artery disease, is the most prevalent type of primary myocardial disease, affecting approximately 1 in 250 persons [1]. It is one of the most frequent causes of congestive heart failure and is the commonest reason for cardiac transplantation in adults and children [2]. In majority of patients, DCM occurs sporadically, but in 25%–35% of cases, familial transmission of DCM is observed in an autosomal dominant, recessive, or X-linked pattern with variable expressivity and penetrance [3]. Increasing evidence demonstrates that genetic defects play an important role in the pathogenesis of DCM, and a great number of mutations in more than 50 genes have been associated with DCM. Of these well established DCM-associated genes, most code for cardiac structural or regulatory proteins, including myocardial sarcomeric and cytoskeletal proteins [1]. However, DCM is a genetically heterogeneous disorder, and the genetic determinants underpinning DCM in a significant proportion of patients remain unknown.

* Corresponding author. Department of Cardiology, Shanghai Chest Hospital, Shanghai Jiao Tong University, 241 West Huaihai Road, Shanghai 200030, China.
** Corresponding author. Department of Cardiology, Shanghai Tenth People's Hospital, Tongji University School of Medicine, 301 Middle Yanchang Road, Shanghai 200072, China.
E-mail addresses: xuyawei69@163.com (Y.-W. Xu), dryyq@tongji.edu.cn (Y.-Q. Yang).

http://dx.doi.org/10.1016/j.bbrc.2015.02.094

It has been found that a group of core cardiac transcription factors, including the homeodomain protein NKX2-5, zinc finger proteins GATA4, GATA5, and GATA6, and T-box factors TBX1, TBX2, TBX3, TBX4, TBX5, TBX18 and TBX20, play a crucial role in the normal cardiovascular development [4]. These core transcription factors physically interact with each other and with an array of other transcription factors, and function in a mutually reinforcing transcriptional network to finely control heart development [4]. Hence, it is not surprising that mutations in most of the core cardiac transcription factors, especially for the most extensively studied cardiac transcription factors NKX2-5, GATA4 and TBX5, have been associated with non-synodromic or synodromic congenital heart diseases as well as arrhythmias [4–22]. Interestingly, mutations in some cardiac transcription factors, such as NKX2-5, GATA4, GATA6 and TBX20, have also been causally linked to DCM in humans [23–28]. Given that the expression profile and functional characteristics of *TBX5* overlap at least partially with those of *NKX2-5*, *GATA4*, *GATA6* and *TBX20* [29–31], it is warranted to make the hypothesis that mutated *TBX5* may contribute to DCM in a subset of patients.

2. Materials and methods

2.1. Study subjects

In this study, a cohort of 190 unrelated patients with DCM was enrolled from the Han Chinese population. The available family members of the index patients were also recruited. A total of 200 ethnically-matched unrelated healthy individuals from a routine physical examination were enlisted as controls. All participants underwent clinical evaluation, chest radiography, electrocardiogram, echocardiography and exercise capacity testing. Cardiac catheterization, coronary angiography, endomyocardial biopsy or cardiac magnetic resonance imaging was performed only if there was a strong clinical indication. The clinical diagnosis of DCM was made in accordance with the criteria established by the World Health Organization/International Society and Federation of Cardiology Task Force on the Classification of Cardiomyopathy: a left ventricular end-diastolic diameter >27 mm/m^2 and an ejection fraction <40% or fractional shortening <25% in the absence of an apparent secondary cause of cardiomyopathy, such as ischemic heart disease, valvular heart disease, essential hypertension, viral myocarditis and cardiac glycogen storage disease [32]. Familial DCM was defined as the DCM occurring in two or more first-degree relatives of a family. This investigation conformed to the principles of the Declaration of Helsinki. The study protocol was reviewed and approved by the local institutional ethics committee. Written informed consent was obtained from all the participants prior to study.

2.2. Screening for TBX5 mutation

Peripheral venous blood samples were taken from all the study participants. Genomic DNA was purified from blood leukocytes of each participant with Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). The referential genomic DNA sequence of *TBX5* derived from GenBank (accession no. NG_007373.1). The primers used to amplify the coding exons and intron/exon boundaries of *TBX5* by polymerase chain reaction (PCR) were designed as shown in Table 1. Amplification of genomic DNA fragment by PCR was carried out using HotStar Taq DNA Polymerase (Qiagen, Hilden, Germany) on a Veriti Thermal Cycler (Applied Biosystems, Foster, CA, USA) with standard conditions and concentrations of reagents. Both strands of each amplicon were sequenced with a BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) under an ABI PRISM 3130 XL DNA Analyzer (Applied Biosystems). For every identified sequence variant, the single nucleotide polymorphism (SNP; http://www.ncbi.nlm.nih.gov/SNP) and Exome Variant Server (EVS; http://evs.gs.washington.edu/EVS) databases were queried to confirm its novelty.

Table 1
Primer pairs used to amplify the coding exons and flanking introns of the *TBX5* gene.

Coding exon	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Size (bp)
1	TGGAAACTGGGGGCCAAACT	TCTGTCCCCGCAAGAGAAGC	385
2	TTGGGGAAGGAATGCCCACT	ATCCAGATAGCACGGCCTCC	470
3	TACCTACAGTTGCCCGCCTG	GATAGGCGGACAGACGCCTT	477
4	CAGTGCGCTACCTCCAGACT	GGTAGAGGCAGAAAGCGACGA	359
5	ACCCTGGCTTTTTCGGTTGG	CACCCTGGGGTCGAAGTTGG	487
6	GGGCAAACCAAACCCAGGTG	GGGACAGAGGGGGCTCATTC	477
7	CACACCTGGTTCAGCCACTC	CACCCCCAACCCAAGGAAAG	369
8	CACTTTTAGCTGCCTGGTGCC	AGGAAATGTCTGTTGTGAAGCAGG	665

2.3. Alignment of multiple TBX5 protein sequences across species

Multiple TBX5 protein sequences from various species were aligned using the online MUSCLE program, version 3.6 (http://www.ncbi.nlm.nih.gov/).

2.4. Expression plasmids and site-directed mutagenesis

Human *TBX5* containing the whole coding sequence was produced by PCR amplification using the human full-length cDNAs prepared previously [12], digested with *Eco*RI and *Not*I, and subsequently cloned into the pcDNA3.1 vector (Invitrogen, Carlsbad, CA, USA). The identified mutation was introduced into the wild-type TBX5-pcDNA3.1 construct by PCR-based site-directed mutagenesis using a QuickChange II XL Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA, USA) and verified by sequencing. The expression plasmids NKX2-5-pEFSA, GATA4-pSSRa and ANF-luciferase reporter (ANF-luc), which contains the 2600-bp 5′-flanking region of the *ANF* gene and expresses the Firefly luciferase, were kind gifts provided by Dr. Ichiro Shiojima at Chiba University School of Medicine, Japan.

2.5. Luciferase reporter gene assays

COS-7 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, and maintained in a humidified atmosphere with 5% CO_2. Lipofectamine® 2000 reagent (Invitrogen) was used for transfection of COS-7 cells at about 80% confluency. The internal control vector pGL4.75 (hRluc/CMV, Promega) expressing the Renilla luciferase was used in transient transfection assays to normalize transfection efficiency. In each transfection, the same amount (0.6 μg) of expression plasmid DNA (wild-type TBX5-pcDNA3.1, NKX2-5-pEFSA, GATA4-pSSRa or mutant TBX5-pcDNA3.1) was used alone or together, in combination with 1.0 μg of ANF-luc and 0.04 μg of pGL4.75. Cells were harvested 48 h after transfection, and the Firefly and Renilla luciferase activities were measured with the Dual-Glo luciferase assay system (Promega). The activity of the *ANF* promoter was presented as fold activation of Firefly luciferase relative to Renilla luciferase. Three independent experiments were performed at minimum for each transfection.

2.6. Statistical analysis

Statistical software package SPSS (version 15.0) was used for statistical analysis. Data are expressed as mean ± SD. Differences between means were compared using the Student's unpaired *t* test. Comparison of the categorical variables between two groups was

Table 2
The baseline clinical characteristics of the study population.

Variables	Patients (n = 190)	Controls (n = 200)
Age (years)	56 ± 12	57 ± 11
Male (%)	92 (48)	100 (50)
Positive family history of DCM (%)	68 (36)	0 (0)
SBP (mmHg)	117 ± 15	120 ± 12
DBP (mmHg)	78 ± 8	80 ± 6
LVEDD (mm)	68 ± 7	46 ± 6
LVESD (mm)	55 ± 8	34 ± 5
LVEF (%)	39 ± 9	63 ± 4
LVFS (%)	18 ± 6	31 ± 6
NYHA function class (%)		
I	39 (21)	NA
II	67 (35)	NA
III	61 (32)	NA
IV	23 (12)	NA

DCM indicates dilated cardiomyopathy; SBP, systolic blood pressure; DBP, diastolic blood pressure; 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; and NA, not applicable.

made by using Fisher's exact test. A two-tailed $p < 0.05$ was considered statistically significant.

3. Results

3.1. Clinical characteristics of the study subjects

In this study, a total of 190 unrelated patients with DCM were clinically investigated in contrast to 200 control individuals. All the patients presented with typical DCM phenotype as described previously [32], and had no apparent secondary cause of DCM. The control individuals had normal echocardiographic results without evidence of structural cardiac diseases. The baseline clinical characteristics of the study population are given in Table 2.

3.2. Identification of a novel TBX5 mutation

Direct PCR-sequencing of the *TBX5* gene led to the identification of a heterozygous mutation in 1 out of 190 unrelated patients with DCM, with a mutational prevalence of roughly 0.53%. Specifically, a substitution of guanine (G) for thymine (T) in the first nucleotide of codon 154 (c.460T > G), predicting the replacement of serine (S) by alanine (A) at amino acid position 154 (p.S154A), was identified in a patient with positive family history of DCM. The DNA sequencing electropherograms showing the detected heterozygous *TBX5* mutation of c.460T > G compared with its control sequence are shown in Fig. 1A. A schematic diagram of TBX5 showing the T-box structural domain and location of the mutation identified in this study is presented in Fig. 1B. The missense mutation was neither found in the control individuals nor reported in the SNP and EVS databases. Genetic screening of the index patient's family members showed that the mutation was present in all the affected living family members, but absent in unaffected family members examined. Analysis of the pedigree revealed that the mutation co-segregated with DCM transmitted in an autosomal dominant mode in the family with complete penetrance. Besides, the proband's two younger sisters (II-6 and II-8) had also secundum atrial septal defect and second-degree atrioventricular conduction block. The pedigree structure of the family is illustrated in Fig. 1C. The

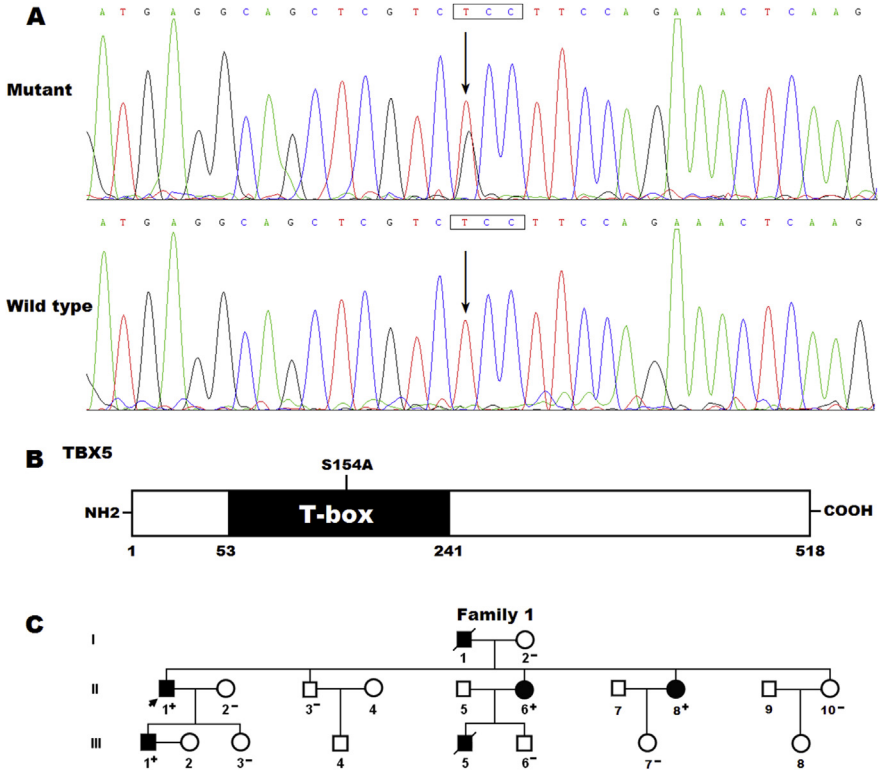

Fig. 1. A novel TBX5 mutation associated with dilated cardiomyopathy. A, sequence electropherograms showing the heterozygous TBX5 mutation compared with its wild-type control. The arrow indicates the heterozygous nucleotides of T/G in the proband (mutant) or the homozygous nucleotides of T/T in the corresponding control individual (wild-type). The rectangle marks the nucleotides comprising a codon of TBX5. B, schematic diagram of TBX5 protein structure with the dilated cardiomyopathy related mutation indicated. The mutation identified in patients with dilated cardiomyopathy is shown above the T-box structural domain. NH_2 denotes amino-terminus; COOH, carboxyl-terminus. C, pedigree structure of the family with dilated cardiomyopathy. Family members are identified by generations and numbers. Square indicates male family member; circle, female member; symbol with a slash, the deceased member; closed symbol, affected member; open symbol, unaffected member; arrow, proband; "+", carrier of the heterozygous missense mutation; and "−", non-carrier.

Table 3
Phenotypic characteristics of the affected pedigree members alive.

Individual	Gender	Age (years)	Cardiac phenotype	LVEDD (mm)	LVESD (mm)	LVEF (%)	LVFS (%)	ECG findings
II-1	M	56	DCM	79	69	34	13	
II-6	F	52	DCM, ASD	64	51	31	19	AVB
II-8	F	49	DCM, ASD	64	50	39	20	AVB
III-1	M	30	DCM	56	43	43	22	

M denotes male; F, female; DCM, dilated cardiomyopathy; ASD, atrial septal defect; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LVEF, left ventricular ejection fraction; LVFS, left ventricular fractional shortening; AVB, atrioventricular conduction block.

phenotypic characteristics of the affected living family members are summarized in Table 3.

3.3. Multiple alignments of TBX5 protein sequences among various species

Alignment of multiple TBX5 protein sequences across species from human to chimpanzee, monkey, dog, cattle, mouse, rat, fowl, zebrafish and frog showed that the altered serine at amino acid residue 154 of TBX5 was completely conserved evolutionarily (Fig. 2).

3.4. Reduced transcriptional activity of the mutant TBX5

As shown in Fig. 3, the same amount (0.6 μg) of wild-type and S154A-mutant *TBX5* constructs transcriptionally activated the *ANF* promoter by ~10-fold and ~3-fold, respectively (wild type *vs* mutant: $t = 11.0480$, $p = 0.0004$), indicating that the S154A-mutant TBX5 has a significantly reduced transcriptional activity compared with its wild-type counterpart.

3.5. Impaired synergistic activation of mutant TBX5 with NKX2-5 or GATA4

As shown in Fig. 3, when the same amount (0.6 μg) of wild-type *NKX2-5* was cotransfected with wild-type or S154A-mutant *TBX5* (0.6 μg), the induced activation of the *ANF* promoter was ~38-fold or ~8-fold (wild type *vs* mutant: $t = 22.8218$, $p = 0.0001$); while in the presence of 0.6 μg of wild-type *GATA4*, the same amount (0.6 μg) of wild-type and S154A-mutant *TBX5* expression plasmids activated the *ANF* promoter by ~22-fold and ~6-fold, respectively (wild type *vs* mutant: $t = 13.5117$, $p = 0.0002$). These results suggest that the mutant TBX5 has an impaired transcriptional activation in synergy with NKX2-5 or GATA4.

4. Discussion

In the present study, a novel heterozygous mutation of p.S154A in TBX5 was identified in a family with DCM. The missense mutation, which was absent in the 400 reference chromosomes from a matched control population, co-segregated with DCM in the family with complete penetrance. A cross-species alignment of multiple TBX5 protein sequences showed that the altered amino acid was completely conserved evolutionarily. Biological assays revealed that the S154A-mutant TBX5 was associated with significantly decreased transcriptional activation alone or in synergy with NKX2-5 or GATA4. Therefore, it is probable that mutated TBX5 contributes to the pathogenesis of DCM in these mutation carriers.

Human *TBX5* is located on chromosome 12q24.1, coding for a protein with 518 amino acids. The TBX5 protein contains an important structural motif called T-box, which is highly conserved throughout members of this family and across species. T-box is required for specific DNA binding and protein–protein interactions, and previous experiments have demonstrated that TBX5 transcriptionally activates multiple target genes expressed during cardiac development, including *ANF*, *CX40* and *SRF*, singly or synergistically with such cooperative partners as NKX2-5, GATA4 and TBX20 [31]. In the current study, the TBX5 mutation identified in DCM patients is located in the T-box and reporter gene analyses showed that the mutant TBX5 was associated with decreased transcriptional activity and diminished synergistic activation with NKX2-5 or GATA4 on a target gene, *ANF*. These functional data suggest that haploinsufficiency or dominant-negative effect caused by TBX5 mutation is likely an alternative pathological mechanism underlying DCM.

The discovery that TBX5 loss-of-function mutation contributes to DCM may be partially ascribed to the developmental defects and remodeling abnormalities of the heart. In humans and vertebrate animals, *TBX5* is highly expressed in the embryonic and adult hearts, playing a pivotal role in cardiac development and structural remodeling, including cell type specification, cardiomyocyte

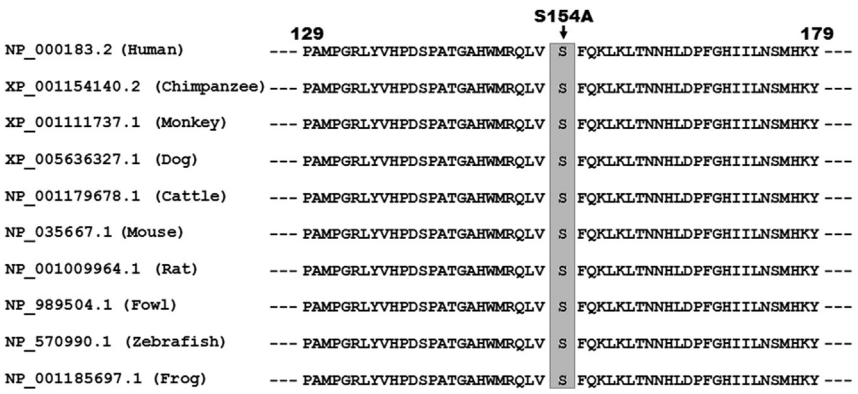

Fig. 2. Alignment of multiple TBX5 protein sequences across species. The altered serine at amino acid position 154 of TBX5 is completely conserved evolutionarily among species.

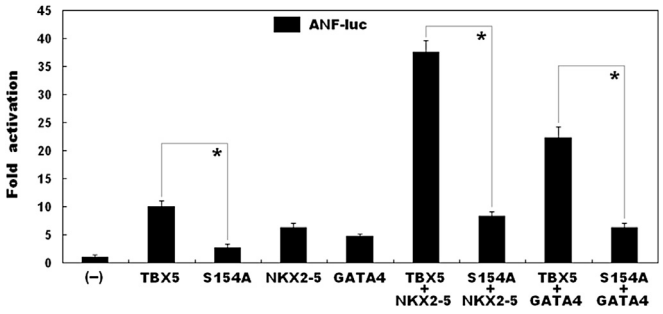

Fig. 3. Functional defects resulted from TBX5 mutation. Activation of atrial natriuretic factor promoter driven luciferase in COS-7 cells by wild-type TBX5 or S154A-mutant TBX5 (S154A), alone or in combination with NKX2-5 or GATA4, showed significantly diminished transcriptional activation by the mutant protein. Experiments were performed in triplicate, and mean and standard deviations are shown. An asterisk * indicates $p < 0.0005$, when compared with wild-type TBX5.

proliferation, myocardial cell differentiation, proepicardial cell migration, tissue patterning and morphogenesis [33]. In mice, *Tbx5* is expressed in 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 [34]. Homozygous *Tbx5*-null mice died by E10.5 mainly due to failure of cardiac looping, hypoplasia of sinuatria and left ventricle. Mice heterozygous for the deletion of Tbx5 allele suffered from atrial septal defects, ventricular septal defects, endocardial cushion defects, hypoplastic left heart, aberrant trabeculation, and atrioventricular block, similar to what were observed in patients with Holt-Oram syndrome [35]. Furthermore, in mice Tbx5 and Gata4 are co-expressed and interact in the developing atria and ventricles, and mice doubly heterozygous for *Tbx5* and *Gata4* displayed embryonic lethality, thin atrial and ventricular myocardium with reduced cell proliferation, and atrioventricular septation defects [36]. Additionally, Tbx5 also physically interacts with other core cardiac transcriptional factors, including Nkx2-5, Mef2c and Tbx20, and forms a transcriptional complex resulting in synergistic activation of multiple downstream genes essential for cardiovascular development, including *Nppa*, *Cx40*, *Srf*, *Mhy6* and *Id2* [31]. Taken together, these experimental results indicate that mutated *TBX5* enhances the susceptibility to DCM in humans.

In addition, loss-of-function mutations in several transcriptional cooperators of TBX5, including NKX2-5, GATA4, GATA6 and TBX20, have been implicated in DCM in humans [23–28]. Therefore, functionally impaired TBX5 probably increases the vulnerability to DCM by reducing synergistic activation of target genes.

Previously, mutations in *TBX5* were reported to cause Holt-Oram syndrome, an autosomal dominant disorder characterized by various cardiac anomalies and anterior upper limb malformations. Cardiac phenotypes comprise a wide spectrum of congenital heart abnormalities including secundum-type atrial septal defect, ventricular septal defect, atrioventricular septal defect, patent ductus arteriosus, tetralogy of Fallot, mitral valve defect, and aberrant pulmonary vein as well as cardiac conduction block and atrial fibrillation, with atrial septal defect being the most common [20,21,37]. Clinically, there are three variations of Holt-Oram syndrome: patients may have only cardiovascular defects (4%), only forelimb deformities (27%), or both (69%), and these cardiac and skeletal malformations vary widely ranging from mild to severe, even within families [21]. In the present study, in addition to atrial septal defect and atrioventricular conduction block, DCM was attributed to a novel TBX5 mutation, thus expanding the phenotypic spectrum associated with TBX5.

In conclusion, this study firstly associates TBX5 loss-of-function mutation with DCM and provides novel insight into the molecular pathogenesis of DCM, suggesting potential implications in development of new preventive and therapeutic strategies for this commonest primary cardiomyopathy.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

We are thankful to the participants for their participation in this study. This work was supported by grants from the National Natural Science Fund of China (81270161, 81470372, 81400244 and 81400180), the key program for Basic Research of Shanghai, China (14JC1405500), the Natural Science Fund of Shanghai, China (13ZR1438400, 14ZR1438000 and 15ZR1438100), the Experimental Animal Program of Shanghai, China (14140903600 and 12140902800), and the Key Project of Shanghai Chest Hospital, China (2014YZDH10102 and 2014YZDH20500).

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.02.094.

References

[1] E.M. McNally, J.R. Golbus, M.J. Puckelwartz, Genetic mutations and mechanisms in dilated cardiomyopathy, J. Clin. Invest. 123 (2013) 19–26.
[2] N.K. Lakdawala, J.R. Winterfield, B.H. Funke, Dilated cardiomyopathy, Circ. Arrhythm. Electrophysiol. 6 (2013) 228–237.
[3] M. Kamisago, S.D. Sharma, S.R. DePalma, S. Solomon, P. Sharma, B. McDonough, L. Smoot, M.P. Mullen, P.K. Woolf, E.D. Wigle, J.G. Seidman, C.E. Seidman, Mutations in sarcomere protein genes as a cause of dilated cardiomyopathy, N. Engl. J. Med. 343 (2000) 1688–1696.
[4] D.J. McCulley, B.L. Black, Transcription factor pathways and congenital heart disease, Curr. Top. Dev. Biol. 100 (2012) 253–277.
[5] X.K. Qu, X.B. Qiu, F. Yuan, J. Wang, C.M. Zhao, X.Y. Liu, X.L. Zhang, R.G. Li, Y.J. Xu, X.M. Hou, W.Y. Fang, X. Liu, Y.Q. Yang, A novel NKX2.5 loss-of-function mutation associated with congenital bicuspid aortic valve, Am. J. Cardiol. 114 (2014) 1891–1895.
[6] W.H. Xie, C. Chang, Y.J. Xu, R.G. Li, X.K. Qu, W.Y. Fang, X. Liu, Y.Q. Yang, Prevalence and spectrum of Nkx2.5 mutations associated with idiopathic atrial fibrillation, Clin. (Sao Paulo) 68 (2013) 777–784.
[7] R.T. Huang, S. Xue, Y.J. Xu, M. Zhou, Y.Q. Yang, A novel NKX2.5 loss-of-function mutation responsible for familial atrial fibrillation, Int. J. Mol. Med. 31 (2013) 1119–1126.
[8] J.L. Perera, N.M. Johnson, D.P. Judge, J.E. Crosson, Novel and highly lethal NKX2.5 missense mutation in a family with sudden death and ventricular arrhythmia, Pediatr. Cardiol. 35 (2014) 1206–1212.
[9] Y.Q. Yang, L. Gharibeh, R.G. Li, Y.F. Xin, J. Wang, Z.M. Liu, X.B. Qiu, Y.J. Xu, L. Xu, X.K. Qu, X. Liu, W.Y. Fang, R.T. Huang, S. Xue, G. Nemer, GATA4 loss-of-function mutations underlie familial tetralogy of fallot, Hum. Mutat. 34 (2013) 1662–1671.
[10] R. Xiang, L.L. Fan, H. Huang, B.B. Cao, X.P. Li, D.Q. Peng, K. Xia, A novel mutation of GATA4 (K319E) is responsible for familial atrial septal defect and pulmonary valve stenosis, Gene 534 (2014) 320–323.
[11] J. Wang, Y.M. Sun, Y.Q. Yang, Mutation spectrum of the GATA4 gene in patients with idiopathic atrial fibrillation, Mol. Biol. Rep. 39 (2012) 8127–8135.
[12] D. Wei, H. Bao, N. Zhou, G.F. Zheng, X.Y. Liu, Y.Q. Yang, GATA5 loss-of-function mutation responsible for the congenital ventriculoseptal defect, Pediatr. Cardiol. 34 (2013) 504–511.
[13] L.M. Shi, J.W. Tao, X.B. Qiu, J. Wang, F. Yuan, L. Xu, H. Liu, R.G. Li, Y.J. Xu, Q. Wang, H.Z. Zheng, X. Li, X.Z. Wang, X.K. Qu, Y.Q. Yang, GATA5 loss-of-function mutations associated with congenital bicuspid aortic valve, Int. J. Mol. Med. 33 (2014) 1219–1226.
[14] R.T. Huang, S. Xue, Y.J. Xu, M. Zhou, Y.Q. Yang, Somatic GATA5 mutations in sporadic tetralogy of Fallot, Int. J. Mol. Med. 33 (2014) 1227–1235.
[15] X.H. Wang, C.X. Huang, Q. Wang, R.G. Li, Y.J. Xu, X. Liu, W.Y. Fang, Y.Q. Yang, A novel GATA5 loss-of-function mutation underlies lone atrial fibrillation, Int. J. Mol. Med. 31 (2013) 43–50.
[16] J. Wang, X.J. Luo, Y.F. Xin, Y. Liu, Z.M. Liu, Q. Wang, R.G. Li, W.Y. Fang, X.Z. Wang, Y.Q. Yang, Novel GATA6 mutations associated with congenital ventricular septal defect or tetralogy of Fallot, DNA Cell Biol. 31 (2012) 1610–1617.

[17] R.T. Huang, S. Xue, Y.J. Xu, Y.Q. Yang, Somatic mutations in the GATA6 gene underlie sporadic tetralogy of Fallot, Int. J. Mol. Med. 31 (2013) 51–58.
[18] J. Li, W.D. Liu, Z.L. Yang, Y.Q. Yang, Novel GATA6 loss-of-function mutation responsible for familial atrial fibrillation, Int. J. Mol. Med. 30 (2012) 783–790.
[19] S. Smemo, L.C. Campos, I.P. Moskowitz, J.E. Krieger, A.C. Pereira, M.A. Nobrega, Regulatory variation in a TBX5 enhancer leads to isolated congenital heart disease, Hum. Mol. Genet. 21 (2012) 3255–3263.
[20] A. Baban, A.V. Postma, M. Marini, G. Trocchio, A. Santilli, M. Pelegrini, P. Sirleto, M. Lerone, S.B. Albanese, P. Barnett, C.J. Boogerd, B. Dallapiccola, M.C. Digilio, R. Ravazzolo, G. Pongiglione, Identification of TBX5 mutations in a series of 94 patients with tetralogy of Fallot, Am. J. Med. Genet. A 164 (2014) 3100–3107.
[21] A.V. Postma, J.B. van de Meerakker, I.B. Mathijssen, P. Barnett, V.M. Christoffels, A. Ilgun, J. Lam, A.A. Wilde, R.H. Lekanne Deprez, A.F. Moorman, A gain-of-function TBX5 mutation is associated with atypical Holt-Oram syndrome and paroxysmal atrial fibrillation, Circ. Res. 102 (2008) 1433–1442.
[22] X. Zang, S. Zhang, Y. Xia, S. Li, F. Fu, X. Li, F. Wang, R. Zhang, X. Tian, L. Gao, J. Zhang, Y. Yang, X. Tu, Q. Wang, SNP rs3825214 in TBX5 is associated with lone atrial fibrillation in Chinese Han population, PLoS One 8 (2013) e64966.
[23] M.W. Costa, G. Guo, O. Wolstein, M. Vale, M.L. Castro, L. Wang, R. Otway, P. Riek, N. Cochrane, M. Furtado, C. Semsarian, R.G. Weintraub, T. Yeoh, C. Hayward, A. Keogh, P. Macdonald, M. Feneley, R.M. Graham, J.G. Seidman, C.E. Seidman, N. Rosenthal, D. Fatkin, R.P. Harvey, Functional characterization of a novel mutation in NKX2-5 associated with congenital heart disease and adult-onset cardiomyopathy, Circ. Cardiovasc. Genet. 6 (2013) 238–247.
[24] F. Yuan, X.B. Qiu, R.G. Li, X.K. Qu, J. Wang, Y.J. Xu, X. Liu, W.Y. Fang, Y.Q. Yang, D.N. Liao, A novel NKX2-5 loss-of-function mutation predisposes to familial dilated cardiomyopathy and arrhythmias, Int. J. Mol. Med. 35 (2015) 478–486.
[25] R.G. Li, L. Li, X.B. Qiu, F. Yuan, L. Xu, X. Li, Y.J. Xu, W.F. Jiang, J.Q. Jiang, X. Liu, W.Y. Fang, M. Zhang, L.Y. Peng, X.K. Qu, Y.Q. Yang, GATA4 loss-of-function mutation underlies familial dilated cardiomyopathy, Biochem. Biophys. Res. Commun. 439 (2013) 591–596.
[26] J. Li, W.D. Liu, Z.L. Yang, F. Yuan, L. Xu, R.G. Li, Y.Q. Yang, Prevalence and spectrum of GATA4 mutations associated with sporadic dilated cardiomyopathy, Gene 548 (2014) 174–181.
[27] L. Xu, L. Zhao, F. Yuan, W.F. Jiang, H. Liu, R.G. Li, Y.J. Xu, M. Zhang, W.Y. Fang, X.K. Qu, Y.Q. Yang, X.B. Qiu, GATA6 loss-of-function mutations contribute to familial dilated cardiomyopathy, Int. J. Mol. Med. 34 (2014) 1315–1322.
[28] E.P. Kirk, M. Sunde, M.W. Costa, S.A. Rankin, O. Wolstein, M.L. Castro, T.L. Butler, C. Hyun, G. Guo, R. Otway, J.P. Mackay, L.B. Waddell, A.D. Cole, C. Hayward, A. Keogh, P. Macdonald, L. Griffiths, D. Fatkin, G.F. Sholler, A.M. Zorn, M.P. Feneley, D.S. Winlaw, R.P. Harvey, 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 (2007) 280–291.
[29] H. Akazawa, I. Komuro, Cardiac transcription factor Csx/Nkx2-5: its role in cardiac development and diseases, Pharmacol. Ther. 107 (2005) 252–268.
[30] S. Pikkarainen, H. Tokola, R. Kerkelä, H. Ruskoaho, GATA transcription factors in the developing and adult heart, Cardiovasc. Res. 63 (2004) 196–207.
[31] F. Greulich, C. Rudat, A. Kispert, Mechanisms of T-box gene function in the developing heart, Cardiovasc. Res. 91 (2011) 212–222.
[32] P. Elliott, C. O'Mahony, P. Syrris, A. Evans, C. Rivera Sorensen, M.N. Sheppard, G. Carr-White, A. Pantazis, W.J. McKenna, Prevalence of desmosomal protein gene mutations in patients with dilated cardiomyopathy, Circ. Cardiovasc. Genet. 3 (2010) 314–322.
[33] T.F. Plageman Jr., K.E. Yutzey, T-box genes and heart development: putting the "T" in heart, Dev. Dyn. 232 (2005) 11–20.
[34] B.G. Bruneau, M. Logan, N. Davis, T. Levi, C.J. Tabin, J.G. Seidman, C.E. Seidman, Chamber-specific cardiac expression of Tbx5 and heart defects in Holt-Oram syndrome, Dev. Biol. 211 (1999) 100–108.
[35] B.G. Bruneau, G. Nemer, J.P. Schmitt, F. Charron, L. Robitaille, S. Caron, D.A. Conner, M. Gessler, M. Nemer, C.E. Seidman, J.G. Seidman, A murine model of Holt-Oram syndrome defines roles of the T-box transcription factor Tbx5 in cardiogenesis and disease, Cell 106 (2001) 709–721.
[36] C. Misra, S.W. Chang, M. Basu, N. Huang, V. Garg, Disruption of myocardial Gata4 and Tbx5 results in defects in cardiomyocyte proliferation and atrioventricular septation, Hum. Mol. Genet. 23 (2014) 5025–5035.
[37] J. Böhm, W. Heinritz, A. Craig, M. Vujic, B.M. Ekman-Joelsson, J. Kohlhase, U. Froster, Functional analysis of the novel TBX5 c.1333delC mutation resulting in an extended TBX5 protein, BMC Med. Genet. 9 (2008) 88.