EXPERIMENTAL STUDY

Phenotype and Functional Analyses in a Transgenic Mouse Model of Left Ventricular Noncompaction Caused by a DTNA Mutation

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Summary

DTNA encoding dystrobrevin-α (α-DB) is a putative causal gene associated with left ventricular noncompaction cardiomyopathy (LVNC). The aim of the study was to investigate the causal role of DTNA in LVNC using a transgenic mouse model.

A missense mutation (c.146A > G, p.N49S) of DTNA was identified in a patient with LVNC by Sanger sequencing. Six independent lines of transgenic mice expressing the mutant DTNA under a myosin heavy chain 6 (Myh6) promoter were generated (Myh6:DTNA<sup>p.N49S</sup>). Phenotypic characteristics of DTNA-p.N49S mutations were evaluated by echocardiography, histological observation, and immunoblotting. Multiple trabeculation and a higher ratio of non-compact to compact myocardial layer were found in the Myh6:DTNA<sup>p.N49S</sup> mice compared to the controls. The transgenic mice also showed left ventricular (LV) dilation and cardiac systolic dysfunction. In conclusion, overexpression of the DTNA-p.N49S mutation in a mouse heart can be responsible for the phenotype of deep trabeculation, dilated cardiomyopathy, and cardiac dysfunction, which resembles the phenotype of LVNC.

Key words: Dystrobrevin-α, Cardiomyopathy, Trabecular, Sanger sequencing

Left ventricular noncompaction cardiomyopathy (LVNC) is a hereditary cardiomyopathy with three defining markers, including prominent left ventricular trabeculations, deep intertrabecular recesses, and a thin compacted layer. The clinical manifestations are usually characterized by thromboembolic events, arrhythmias, heart failure, and sudden death. A myocardial development arrest, which hinders compaction of the loose myocardial meshwork, could be the potential pathogenesis. Several putative causal genes associated with LVNC have been identified including TAZ,6 LDB3,5 DTNA,6 FKBP1,7 LMN,2 MBL1,5 TNNT2,5 MYH7,2,5 and ACTC1.2

Alpha-DB (α-DB) encoded by the DTNA gene is a scaffold unit structure for signaling molecules at the sarcolemma of cardiac muscle. It is involved in maintaining the structural integrity of muscle fibers by linking the extracellular matrix to the subsarcomembranous cytoskeleton. In the present study, we identified a novel missense mutation (p.N49S) in exon 4 (c.AAT > AGT) of DTNA in a patient with LVNC. To determine the causal role of DTNA-p.N49S mutation in the pathogenesis of LVNC, we generated and characterized a transgenic mouse model expressing the DTNA<sup>p.N49S</sup> mutant specifically in the heart.

Methods

The animal studies were approved by the Animal Ethics and Experimentation Committee of Nanchang University, and performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Mutation screening: The research protocol for humans was reviewed and approved by the Ethical Committee at the regional institute. Thirty-two unrelated patients with a diagnosis of LVNC were studied. All subjects were clinically evaluated by two-dimensional and Doppler echocardiography and electrocardiography (ECG). Blood

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samples were drawn after informed consent was signed. Genomic DNA was extracted from peripheral lymphocytes. All coding exons of LVNC candidate genes (TAZ, LDB3, FKB1, DTA, LMN, MBI, TNNT2, MYH7, and ACTC1) were amplified by polymerase chain reaction (PCR) and sequenced by the Sanger method using a Big Dye Terminator Mix (Applied Biosystems, Carlsbad, CA, USA) in both strands using an ABI PRISM 3130 Automatic DNA Sequencer (Applied Biosystems).\(^{16}\)

**Site-directed mutagenesis:** The plasmid pSK-RSV-m87k (16.1)-DTNA containing the mus Dtna cDNA clone was a gift from Margaret M. Mainone\(^{16}\) (SUNY Upstate Medical University, NY, USA). The A→G substitution at the coding position 146 (p.N49S) was induced by oligonucleotide-mediated site-directed mutagenesis (Quick-Change II XL Site-Directed Mutagenesis Kit, Agilent Technologies, Inc.) using the mutagenic oligonucleotide primers: 5′-ggaagaatgcaGttgcacctgtgtgcagat-3′ and 5′-accagggtgcaaatgcagcagacgacagatag-3′ and S′-accagggtgcaaatgcagcagacgacagatag-3′.

**Generation of transgenic mouse lines:** Mice strains B6 CBF1, C57Bl/6, expression vector pMyh6 and pInsulator were from a Model Animal Research Center of Nanjing University in China. The entire transgenic expression cassette, comprised of the Myh6 promoter and the mus Dtna\(^{16}\) cDNAs, were excised from the transgenic plasmids, purified and injected into the pronucleus of fertilized zygotes harvested from the B6CBF1 mice. Founder mice were generated per conventional methods.\(^{16}\) Genotyping of transgenic mice was performed by PCR using the primers S′-GTTCCTCCTGTAGACGACGATCAC-3′ and S′-TTCCGGCAATGCTTCAATGACGTTC-3′. Quantitative transgene copy number analyses were performed by qPCR using the SYBR\(^\text{⡴}\) Dye Terminator Mix (Applied Biosystems, Carlsbad, CA, USA). The A → G substitution at the coding position 146 (p.N49S) was induced by oligonucleotide-mediated site-directed mutagenesis (Quick-Change II XL Site-Directed Mutagenesis Kit, Agilent Technologies, Inc.) using the mutagenic oligonucleotide primers: 5′-ggaagaatgcaGttgcacctgtgtgcagat-3′ and 5′-accagggtgcaaatgcagcagacgacagatag-3′ and S′-accagggtgcaaatgcagcagacgacagatag-3′. Real-time PCR: Total RNA was prepared from cells using TRIzol reagent (Invitrogen). First-strand cDNA synthesis was performed using PrimeScript\textsuperscript{⡴} RT reagent Kit with gDNA Eraser (TAKARA) according to the manufacturer’s instructions. Real-time-PCR for the expression of rat atrial natriuretic factor (ANF) and brain natriuretic peptide (BNP) were performed using SYBR\textsuperscript{⡴} Premix Ex Taq\textsuperscript{⡴} II (Tli RNaseH Plus) and a ROX plus kit (TAKARA), and the relative levels of expression were normalized to glyceraldehyde-phosphate dehydrogenase (GAPDH) with the ΔΔCT method. The primers for the real-time PCR assay were as follows: forward/reverse primers S′-GATGATTCTGCTCCTGCTTTT-3′/5′-GCCAT

**Histological studies:** Myocardial cross-sections from 2 or 4 mm in thickness were cut throughout the left and the right ventricles. Sections were fixed in formalin and embedded in paraffin and stained with hematoxylin and eosin (H&E) and Masson trichrome stained.

**Measurement of compact myocardium:** Olympus’s Cell-Sens Ver1.5 imaging software was used to measure the thickness of the compact myocardium and the trabecular myocardium area. The compact myocardium thickness was measured in six evenly spaced regions along the perimeter of the ventricular walls;\(^{16}\) for measurement of the trabecular thickness, the software delineated the boundary of the myocardium and measured ventricular wall thickness, subtracting thickness of the compact myocardium.

**Plasmid construction, cell culture, and transfection:** The full-length cDNA of Dtna\(^{16}\) and Dtna\(^{16}\) were amplified by PCR, and were subcloned into the pRES2-ZsGreen1 vector respectively (Yingrun Biotechnologies Inc.). H9C2 rat cardiomyoblast cells were obtained from ATCC, and cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. Cells were plated onto 6-cm culture dishes and cultured overnight before gene transfection. A total amount of 10 μg of empty vector, pRES2-ZsGreen1-Dtna\(^{16}\) and pRES2-ZsGreen1-Dtna\(^{16}\) were transfected into H9C2, respectively, using Lipofectamine 2000 reagent (Invitrogen) following the manufacturer’s protocol. After an additional 48 hours, cells were harvested for immunoprecipitation or PCR assay.

**Real-time PCR:** Total RNA was prepared from cells using TRIzol reagent (Invitrogen). First-strand cDNA synthesis was performed using PrimeScript\textsuperscript{⡴} RT reagent Kit with gDNA Eraser (TAKARA) according to the manufacturer’s instructions. Real-time-PCR for the expression of rat atrial natriuretic factor (ANF) and brain natriuretic peptide (BNP) were performed using SYBR\textsuperscript{⡴} Premix Ex Taq\textsuperscript{⡴} II (Tli RNaseH Plus) and a ROX plus kit (TAKARA), and the relative levels of expression were normalized to glyceraldehyde-phosphate dehydrogenase (GAPDH) with the ΔΔCT method. The primers for the real-time PCR assay were as follows: forward/reverse primers S′-GATGATTCTGCTCCTGCTTTT-3′/5′-GCCAT

**Western blotting:** Protein was extracted from homogenized heart tissues and the cultured H9C2 cell line. The protein concentration was determined using a bicinchoninic acid (BCA) protein assay method. Aliquots of 100 μg protein extracts were loaded on a polyacrylamide gel, subjected to electrophoresis, and transferred to a PVDF membrane. Bands on membranes were detected by chemiluminescence (ECL) detection system (Thermo Fisher Scientific) and bands on membranes were visualized with chemiluminescence (ChemilDoc XRS+, BioRad). All western blots are representative of at least 3
the WW domain of B). The variant affects a highly conserved nucleotide in asparagine at position 49 (p.N49S) (Figure 1, A and B). Sequence in unrelated control individuals. DTNA transition at coding nucleotide 146 (c.AAT > AGT) in We identified a heterozygous missense mutation A > G gene was performed in 32 independent LVNC patients. as well. In all patients, nucleotide sequences of other can- 

Sequencing Project database, and 1,000 Genomes database had the same ethnic background, the NHLBI GO Exome diagnosis for serine at position 49 (p.N49S) (Figure 1, A and DTNA- 

The shaded serine (S) indicates the conserved residue across different species. The missense mutation p.N49S affecting the conserved serine residue in human DTNA is indicated by an arrow. Species abbreviations are as follows: Hs, Homo sapiens; Fc, Felis catus; Dr, Danio rerio; Mm, Mus musculus; D: Transthoracic echocardiography showed prominent trabeculations, with deep intertrabecular recesses in the apex of the left ventricle. There was an increase in LV wall thickness, with diffuse hypokinesia and systolic dysfunction (EF 34%). Ratio of non-compacted layer (17 mm) /compacted layer (7 mm) > 2. E: Electrocardiography (ECG) demonstrating signs of a first-degree atrioventricular block.

Figure 1. Mutation in human DTNA causing LVNC. A: An asparagine-to-serine missense mutation (N49S) in DTNA was found in one patient with LVNC. N, asparagine; S, serine (B). Sequence in unrelated control individuals. C: Evolutionary conservation of Asn49. Partial amino acid sequence alignment of human DTNA with other orthologs. The shaded serine (S) indicates the conserved residue across different species. The missense mutation p.N49S affecting the conserved serine residue in human DTNA is indicated by an arrow. Species abbreviations are as follows: Hs, Homo sapiens; Fc, Felis catus; Dr, Danio rerio; Mm, Mus musculus; D: Transthoracic echocardiography showed prominent trabeculations, with deep intertrabecular recesses in the apex of the left ventricle. There was an increase in LV wall thickness, with diffuse hypokinesia and systolic dysfunction (EF 34%). Ratio of non-compacted layer (17 mm) /compacted layer (7 mm) > 2. E: Electrocardiography (ECG) demonstrating signs of a first-degree atrioventricular block.

Results

**Patient characteristics:** A total of 32 independent cases with LVNC were included in this study. No genetic etiology can be defined in these patients. The diagnosis of LVNC was made according to the Jennie Criteria (2001), which was confirmed by echocardiography.19)

**Mutation identification:** Direct sequencing of the DTNA gene was performed in 32 independent LVNC patients. We identified a heterozygous missense mutation A > G transition at coding nucleotide 146 (c.AAT > AGT) in DTNA exon 4, which resulted in the substitution of asparagine for serine at position 49 (p.N49S) (Figure 1, A and B). The variant affects a highly conserved nucleotide in the WW domain of α-DB (Figure 1C). The absence of DTNA-p.N49S was found in the 400 healthy controls who had the same ethnic background, the NHLBI GO Exome Sequencing Project database, and 1,000 Genomes database as well. In all patients, nucleotide sequences of other can- 

didate genes of LVNC including TAZ, LDB3, FKBP1, LMN, MIB1, TNNT2, MYH7, and ACTC1 were analyzed, and no additional mutations were found.

**Clinical phenotype of the mutation carrier:** This patient was a 39-year-old man who was referred for evaluation of exercise-related chest distress and palpitation for 8 years without a family history of sudden cardiac death. Transthoracic echocardiography showed prominent trabeculations and intertrabecular recesses in the apex of the left ventricle, with a 17 mm uncompacted layer vs. a 7 mm compacted layer. The end-diastolic diameter of the left ventricle was 80 mm. Diffuse hypokinesia was present with a LVEF of 34% (Figure 1D). A first-degree atrioventricular block was shown by ECG (Figure 1E). Based on his symptoms and the echocardiographic findings, the patient was diagnosed with noncompaction of the ventricular myocardium. Heart transplantation was recommended, but he refused it for personal reasons. Several medications were used to treat the patient, including digoxin (0.125 mg, qd), antisterone (20 mg, qd), hydrochlorothiazide (50 mg, qd), furosemide (40 mg, qd), aspirin (100 mg, qd), captopril (12.5 mg, tid), carvedilol (12.5 mg, bid), and potassium chloride sustained-release tablets (1 g tid). He was discharged from the hospital after improvement of his clinical symptoms.

**Cardiac phenotypic characterization of the Dtna**




further validate the pathogenic effects of the DTNA-p.N49S mutation \textit{in vivo}, transgenic mice were generated with cardiac-specific overexpression of mouse $\alpha$-DB protein harboring the p.N49S mutation ($Dtna^{\text{p.N49S}}$). Stable overexpression of the $\alpha$-DB was detected in the heart tissue of transgenic mice (Figure 2, A and B). Increased expression level of BNP, a marker of myocardial hypertrophy and heart failure, was detected by western blotting in the $Dtna^{\text{p.N49S}}$ mice (Figure 2, C and D). There was no significant difference among the wildtype, littermate controls, or $Dtna^{\text{N49S}}$ group in the expression level of myosin light chain 2 (MLC2) (Figure 2, E and F).

Echocardiographic findings of 4-month-old $Dtna^{\text{N49S}}$ mice and controls are summarized in Table I. Compared with littermate controls and wild type mice, LV posterior wall thickness (LVPWT) was significantly increased in the $Dtna^{\text{N49S}}$ mice group (Figure 3, A-F). LV systolic and diastolic function remained stable in this age range. (Table I). Multi-trabeculation was noted in the non-compacted layer of the LV apex cordis among 3 of 15 4-month-old $Dtna^{\text{N49S}}$ mice (Figure 3G). Compared with controls (Figures 3H, 3I), looser myofibers in the compact layer and higher noncompaction index (a ratio of non-compacted myocardium to compact myocardium) were found in the $Dtna^{\text{N49S}}$ mice (Figure 3J). In addition, there were no significant fibrosis changes in all groups of mice myocytes (Figure 3, K-M).

The difference in echocardiographic data was even more remarkable for 8-month-old mice as shown in Table II. Dilated LV and thinner LV walls were detected in the 8-month-old $Dtna^{\text{N49S}}$ mice group, with lower LVEF and LVFS. There were no significant differences in diastolic LV diameter, volume, and stroke volume (Table II). Moreover, there was no difference in 1-year survival rate among the $Dtna^{\text{N49S}}$ mice, wildtype mice, and littermate controls (data not shown).

To elucidate the effects of $Dtna^{\text{N49S}}$ on the cardiac phenotype, we eliminated the influence of wild type $Dtna$ overexpression. The expression levels of $Anf$, $Bnp$, and $Myh7$ were detected \textit{in vitro}. The $p$RES2-ZsGreen1-Dtna\textsuperscript{N49S} and $p$RES2-ZsGreen1-Dtna were transfected into H9C2 (the rat cardiomyoblast cell line) and the control group was cells transfected with empty vector plasmid. Forty-eight hours after the transfection, the expression of DTNA and MYH7 were detected by western blot, and the expression of $Anf$ and $Bnp$ were analyzed by quantitative real-time PCR (qRT-PCR). Compared to the vector group, the protein level of DTNA in $Dtna^{\text{N49S}}$ and $Dtna^{\text{N49S}}$ was...
overexpressed by an average of 1.7-fold and 1.6-fold, respectively (Figure 4, A and B), which indicated that the plasmids were transfected into cells effectively. In addition, there were similar levels of expression of DTNA in the two overexpression groups. Expression of DTNA\textsuperscript{N49S} in H9C2 was associated with a slight but significant up-regulation of Myh7; however, the expression of Myh7 was not affected in the Dtnawt group (Figure 4, A and B).

Following DTNA\textsuperscript{N49S} overexpression, Anf and Bnp mRNA expression were down-regulated significantly (Figure 4, C). However, compared to the DTNA\textsuperscript{N49S} group, Anf expression in the DTNA\textsuperscript{N49S} group was increased by an average of 37.2\% (\( P < 0.05 \)) (Figure 4, C). DTNA\textsuperscript{N49S} also induced an increased mRNA expression level of Bnp, but this trend had no statistical significance (Figure 4, C).

**Discussion**

In this study, a missense mutation (p.N49S) of the DTNA gene was found in one of 32 LVNC patients by genetic screening. Then, a cardiac-specific transgenic mouse model that overexpressed mutant DTNA was established to study the phenotypes of DTNA\textsuperscript{N49S}. A progressive cardiomyopathy characterized by dilated and thinner LV, cardiac systolic dysfunction, and age-related LV hypertrabeculation was found in our transgenic mouse model.

In previous studies focusing on the establishment of genetically modified mice associated with LVNC candidate genes,\textsuperscript{9,20-23} the notch signaling pathway has been found to play a vital role in the development of LVNC. Cardiac-specific inactive Mib1 (an E3 ubiquitin ligase of NOTCH ligands) in mice could cause LVNC by disrupting the Notch pathway.\textsuperscript{9} Another study has demonstrated that Tafazzin knockdown mice showed myocardial hypertrabeculation-noncompaction and lower proliferation rates were identified in trabecular myocardium than compact myocardium.\textsuperscript{9} However, some other studies failed to obtain a definite LVNC phenotype based on genetic mouse models. In the transgenic mice expressing the LVNC-causing mutation E96K in the cardiac troponin T gene (TNNT2), a typical phenotype of DCM (dilated cardiomyopathy) was observed without any sign of LVNC.\textsuperscript{14}

In 2003, mutations in the Cypher/ZASP gene (e.g., p.S196L, etc.) were found to be associated with DCM and LVNC.\textsuperscript{9} Nevertheless, neither cardiac-specific Cypher knockout mice nor the transgenic mice with cardiac-specific expression of the ZASP-p.S196L mutation showed the expected phenotype of LVNC.\textsuperscript{22,23} Therefore, only various parts of mouse models can effectively reproduce the LVNC phenotype. These results suggest that the different genetic background between mice and humans may affect the development of LVNC. LVNC is a complicated and variable cardiomyopathy of which the precise etiology and underlying mechanisms need to be further studied.

In the present study, mild hypertrabeculation could be found in the non-compacted layer of the LV wall close to the apex cordis in some of the DTNA\textsuperscript{N49S} mice. Compared with littermate controls and wild type mice, the noncompaction index was higher in DTNA\textsuperscript{N49S} LVPWT in 4-month-old DTNA\textsuperscript{N49S} mice than in controls. Dilated ventricular wall and decreased myocardium contractile function appeared in the 8-month-old DTNA\textsuperscript{N49S} mice group. It is interesting that the LV structural phenotypes of DTNA\textsuperscript{N49S} mice exhibited a dynamic change like a swollen balloon. With aging, DTNA\textsuperscript{N49S} mice presented the signs of a thinner ventricular wall, a dilated ventricular chamber, and impaired cardiac ejection. In a previous study, a DTNA mutation (P121L) was described, and it resulted in a dilated hypertrophic cardiomyopathy with deep trabeculations, associated with congenital heart diseases.\textsuperscript{24} Our data from a murine model also support the hypothesis that a DTNA deficiency is associated with cardiac structural abnormalities including LVNC.

LVNC-related pathogenic genes have been found to overlap with other cardiomyopathies (hypertrophic cardiomyopathy, DCM or some systemic myopathies). Indeed, the phenotypical overlap and the diversity of clinical manifestations have resulted in a dispute as to whether

**Table 1.** Echocardiographic Indices in 4 Month Old Male Mice

| Indices            | DTNA\textsuperscript{N49S} n = 13 | Littermate controls n = 14 | Wild type n = 11 |
|--------------------|----------------------------------|---------------------------|------------------|
| Body Weight (g)    | 21.36 ± 2.57                     | 21.67 ± 2.62              | 23.23 ± 3.71     |
| Heart Rate (bpm)   | 512.30 ± 34.67                   | 505.31 ± 30.97            | 506.52 ± 42.41   |
| LVAW-ED (mm)       | 0.91 ± 0.11                      | 0.84 ± 0.10               | 0.83 ± 0.15      |
| LVPWT-ED (mm)      | 0.86 ± 0.08**\(\Delta\Delta\)     | 0.70 ± 0.06**             | 0.71 ± 0.06**\(\Delta\Delta\) |
| LVPWT-ES (mm)      | 1.21 ± 0.14**\(\Delta\Delta\)     | 1.05 ± 0.11**             | 1.08 ± 0.12**\(\Delta\Delta\) |
| LVESD (mm)         | 2.34 ± 0.27                      | 2.32 ± 0.28               | 2.32 ± 0.35      |
| LVEDD (mm)         | 3.51 ± 0.31                      | 3.54 ± 0.21               | 3.64 ± 0.29      |
| LVSV (µl)          | 19.48 ± 5.66                     | 19.08 ± 5.61              | 19.23 ± 7.18     |
| LVDD (µl)          | 52.00 ± 11.16                    | 52.59 ± 7.44              | 56.53 ± 10.64    |
| EF (%)             | 61.53 ± 12.14                    | 63.78 ± 9.28              | 66.54 ± 9.69     |
| FS (%)             | 32.96 ± 8.06                     | 34.35 ± 6.75              | 36.49 ± 6.77     |

LVAW-ED indicates left ventricular end-diastolic anterior wall thickness; LVPWT-ED, left ventricular end-diastolic anterior wall thickness; LVPWT-ES, left ventricular end-systolic anterior wall thickness; LVESD, left ventricular end-systolic diameters; LVEDD, left ventricular end-diastolic diameters; LVSV, left ventricular systolic volume; LVDD, left ventricular diastolic volume; EF, percent ejection fraction; FS, percent fractional shortening; and DTNA\textsuperscript{N49S}, DTNA\textsuperscript{N49S} transgenic mice. **\(P < 0.01\) between DTNA\textsuperscript{N49S} and littermates; \(\Delta\Delta\) \(P < 0.01\) between DTNA\textsuperscript{N49S} and wild type mice.
Table II. Echocardiographic Indices in 8 Month Old Male Mice

| Indices               | \(Dtna^{49S}\) \(n=12\) | Littermate controls \(n=10\) | Wild type \(n=10\) |
|-----------------------|--------------------------|----------------------------|-------------------|
| Body Weight (g)       | 25.24 ± 2.13             | 26.70 ± 3.26               | 26.15 ± 2.97      |
| Heart Rate (bpm)      | 481.63 ± 46.79           | 461.42 ± 37.31             | 474.33 ± 41.74    |
| LVAW-ED (mm)          | 0.89 ± 0.14              | 0.86 ± 0.12                | 0.85 ± 0.10       |
| LVDPWT-ED (mm)        | 0.70 ± 0.13              | 0.76 ± 0.07                | 0.85 ± 0.15       |
| LVDPWT-ES (mm)        | 1.06 ± 0.16**△△          | 1.32 ± 0.14**              | 1.39 ± 0.18△△     |
| LVESD (mm)            | 2.72 ± 0.28**△△          | 2.15 ± 0.27**              | 2.14 ± 0.41△△     |
| LVEDD (mm)            | 3.88 ± 0.33              | 3.65 ± 0.17                | 3.64 ± 0.57       |
| LVSV (μl)             | 28.11 ± 2.22**△△         | 15.72 ± 1.15**             | 16.57 ± 2.35△△    |
| LVDV (μl)             | 65.84 ± 13.51            | 56.60 ± 6.40               | 57.89 ± 18.94     |
| EF (%)                | 57.57 ± 4.35**△△         | 72.63 ± 6.44**             | 73.33 ± 7.55**△△  |
| FS (%)                | 29.87 ± 2.89**△△         | 41.28 ± 5.43**             | 42.04 ± 6.47△△    |

LVAW-ED indicates left ventricular end-diastolic anterior wall thickness; LVDPWT-ED, left ventricular end-diastolic posterior wall thickness; LVDPWT-ES, left ventricular end-systolic posterior wall thickness; LVESD, left ventricular end-systolic diameters; LVEDD, left ventricular end-diastolic diameters; LVSV, left ventricular systolic volume; LVDV, left ventricular diastolic volume; EF%, percent ejection fraction; FS%, percent fractional shortening; and \(Dtna^{49S}\), \(Dtna^{49S}\) transgenic mice. **P < 0.01 & *P < 0.05 between \(Dtna^{49S}\) and Littermates; △△P < 0.01 & △P < 0.05 between \(Dtna^{49S}\) and Wild type.
LVNC is a unique cardiomyopathy or only a morphologic trait in various cardiomyopathies.\(^5\) Our results also indicate that this gene mutation may not be the decisive factor for the development of cardiac noncompaction. In our murine model study, 4-month-old DtnaN49S mice were characterized by ventricular trabeculations. Nevertheless, the manifestation of ventricular trabeculations in 8-month-old mice became less apparent. According to our findings, the cardiac structural remodeling developed continuously in the adult mice and finally led to an irreversible impaired cardiac function. In this case, it is difficult to confirm the reason why high ventricular trabeculations had such a variation tendency. However, there are two possible explanations. First, the ventricular trabeculations did not disappear, and it became unlikely for them to be observed due to the diminished thickness and dilation of the LV wall. Like the trabecular remodeling during development of LV in a mammalian embryonic heart, the compaction process (the compacted myocardium layer gets thicker and the non-compacted myocardium layer gets thinner) coincides with the increase in ventricular volumes.\(^25\) In our study, the attenuation of ventricular trabeculations is associated with the particular morphological remodeling of LV. The whole layer of the LV wall became thinner; however, this change of the non-compact layer was more severe than that of the compact layer, and as a result the noncompaction index of DtnaN49S mice was decreased.

Second, hypertrabeculation might be one of the specific morphological traits in this complex cardiomyopathy induced by genetic heterogeneity and variability of hereditary patterns.

A down-regulation of MLC2 encoded by the MYL2 gene has been shown to be associated with the severity of the chronic heart failure.\(^26,27\) In our *in vivo* study, a declining trend of MLC2 expression level was observed. BNP, one of the biomarkers in early myocardial hypertrophy and heart failure\(^28\) was highly up-regulated in ventricle of DtnaN49S mice. In our further *in vitro* study, the expression of cardiac hypertrophic markers (ANF and MYH7) was significantly induced by DtnaN49S mutants when compared to the wild type Dtna transfection group. These data suggested a critical role of DtnaN49S mutations in myocardial hypertrophy and LV dysfunction, which are not caused by an excessive protein expression level of DTNA. Interestingly, wild type DTNA overexpression in a H9C2 cell line repressed the transcription of Anf and Bnp, which implies that DTNA might be associated with the regulation pathways of Anf and Bnp, and moreover plays a protective role in cardiac function.

Although, genetically, it has been shown that DTNA mutations are associated with LVNC cases,\(^4,6\) at present, the data on its role in the pathogenesis of LVNC are limited. Normal cardiomyocytes proliferation mediated by the Notch signaling pathway during heart development plays
a key role in trabecular maturation and compaction.9,20) Disrupting Notch signaling cause developmental arrest in ventricular maturation and myocardial compaction, which leads to LVNC.9,20)

Alpha-DB encoded by DTNA belongs to the cytoskeletal protein family. Dystrobrevin, a component of the dystrophin-associated protein complex (DAPC), is localized in the sarcolemma and serves as a bridge between cytoskeletal and the extracellular matrix.21) The mutation N49S is located in the EF-hand type1 domain of α-DB, which provides specificity in beta-dystroglycan recognition and stabilization of the WW domain to enhance DAPC binding.31) In DAPC, α-DB could help to biochemically stabilize the interaction of dystrophin with the membrane bound dystroglycan complex in skeletal muscle cells and cardiomyocytes.22,31) DAPC was reported to be associated with cell proliferation pathways including Notch, BMP, and Neuregulin 3 signaling pathways in muscle satellite cells.31) DtnaN49S may affect the stability of DAPC, which causes the dysregulation of several downstream signaling pathways related to cell proliferation. We hypothesize that this may be the underlying mechanism of LVNC caused by Dtna.30) The α-DB deficiency may lead to destruction of the integrity of sarcolemma and high susceptibility to injury during cardiac stress induced by a high dose isoproterenol challenge.26) It is noteworthy that, although DTNA is considered to be one of the LVNC-causing genes, the hearts of Dtna knockout mice do not show any features of ventricular noncompaction without any artificial stimuli.26) These data also indicate that catecholamine content might be thought of a considerable factor for the development of LVNC in a genetic background of Dtna mutation.

Conclusions

In conclusion, we identified a DTNA mutation, N49S, in a LVNC patient. Moreover, the phenotypes that we have found in a transgenic mouse model of this Dtna mutation were consistent with the development of cardiomyopathy characterized by LV dysfunction. Although we failed to find an obvious performance of LV trabecular and the mired trabecular fossae in the ventricular myocardium of Dtna mice during different ages, the ratio of non-compacted myocardium to compact myocardium was higher in 4-month-old mice, indicating a phenotype of hypertrabeculation. These data suggest that variants in susceptibility genes might have combined effects with environmental factors on the pathogenesis of LVNC.

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Disclosures

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