Clinical and Echocardiographic Impact of Tafazzin Variants on Dilated Cardiomyopathy Phenotype in Left Ventricular Non-Compaction Patients in Early Infancy

Keiichi Hirono, MD, PhD; Yukiko Hata, PhD; Makoto Nakazawa, MD, PhD; Nobuo Momoi, MD, PhD; Tohru Tsuji, MD, PhD; Taro Matsuoka, MD, PhD; Mamoru Ayusawa, MD, PhD; Yuriko Abe, MD, PhD; Tamaki Hayashi, MD, PhD; Nobuyuki Tsuji, MD; Tadaaki Abe, MD, PhD; Heima Sakaguchi, MD, PhD; Ce Wang, MD; Asami Takasaki, MD, PhD; Shinya Takarada, MD; Mako Okabe, MD; Nariaki Miyao, MD; Hideyuki Nakaoka, MD, PhD; Keijiro Ibuki, MD, PhD; Kazuyoshi Saito, MD, PhD; Sayaka Ozawa, MD, PhD; Naoki Nishida, MD, PhD; Neil E. Bowles, PhD; Fukiko Ichida, MD, PhD

Background: Left ventricular non-compaction (LVNC) is a cardiomyopathy morphologically characterized by 2-layered myocardium and numerous prominent trabeculations, and is often associated with dilated cardiomyopathy (DCM). Variants in the gene encoding tafazzin (TAZ) may change mitochondrial function and cause dysfunction of many organs, but they also contribute to the DCM phenotype in LVNC, and the clinical and echocardiographic features of children with this phenotype are poorly understood.

Methods and Results: We enrolled 92 DCM phenotype LVNC patients and performed next-generation sequencing to identify the genetic etiology. Ten TAZ variants were identified in 15 male patients (16.3%) of the 92 patients, including 3 novel missense substitutions. The patients with TAZ variants had a higher frequency of early onset of disease (92.3% vs. 62.3%, P=0.0182), positive family history (73.3% vs. 20.8%, P=0.0001), and higher LV posterior wall thickness Z-score (8.55±2.60 vs. 5.81±2.56, P=0.0103) than those without TAZ variants, although the mortality of both groups was similar.

Conclusions: This study provides new insight into the impact of DCM phenotype LVNC and emphasizes the clinical advantages available for LVNC patients with TAZ variants.

Key Words: Cardiomyopathy; Left ventricular non-compaction; Tafazzin (TAZ)

Left ventricular non-compaction (LVNC), a recently classified form of inherited cardiomyopathy, is morphologically characterized by a 2-layered myocardium, numerous prominent trabeculations, and deep intertrabecular recesses communicating with the LV cavity. The clinical manifestations are highly variable, ranging from no symptoms to a progressive deterioration that results in congestive heart failure, arrhythmia, thrombosis, and sudden death. To date, variants in several genes, including the gene encoding tafazzin (TAZ), have been reported in LVNC patients, but the relatively small contribution of these known variants to the disease suggests that variants in other genes remain to be identified.

TAZ, also known as G4.5, is located on the long q arm region of chromosome Xq28, encodes for tafazzin, and its genetic variants are responsible for Barth syndrome (BTHS; OMIM 302060). It consists of 11 exons, the first 2 of which are non-coding, and spans approximately 11kb. Although LVNC is frequently described in BTHS, the clinical and echocardiographic features of LVNC with TAZ variants in children are poorly understood.

LVNC is often seen as a distinct phenotype, although it...
was originally described in association with congenital heart disease. In a recent study, patients with the dilated cardiomyopathy (DCM) phenotype LVNC had a higher mortality and severe cardiac events than those with other phenotypes. The natural history of DCM phenotype LVNC due to TAZ variants in children is poorly understood.\(^1\)

In this study, in order to analyze genotype-phenotype correlations in patients with DCM phenotype LVNC and TAZ variants, we screened 92 Japanese LVNC patients for variants in TAZ using next-generation sequencing (NGS) and identified specific manifestations from clinical and echocardiographic data.

**Methods**

**Subjects**

Between 1998 and 2017, Japanese probands with LVNC were referred to Toyama University Hospital for genetic testing from multiple Japanese hospitals. Patients <18 years old, in whom a recent diagnosis of LVNC was made at participating institutions, were eligible for inclusion. Children with secondary etiologies of cardiomyopathy, such as pulmonary disease, endocrine disease, rheumatic disease, immunologic disease, cardiotoxic exposure, or systemic hypertension, children with pacemaker due to arrhythmia, and children without follow-up records were excluded.

Once a proband was identified, a family history was obtained, and all potentially informative family members underwent physical examination, chest radiograph, electrocardiogram (EKG), and echocardiogram. Outcome data collected included physiologic consequences, rhythm disturbances, death, and heart transplantation.

Heart failure was defined either by its notation in the medical record by the treating cardiologist or by the New York Heart Association or Ross classifications present in the medical record. In addition, laboratory data were recorded. Neutropenia was defined as absolute neutrophil count (ANC) <1.5x10^9/L. Sudden death was defined as unexpected death occurring ≤1 h after the onset of a symptomatic event.

Informed consent was obtained from all participants, according to institutional guidelines. This study protocol conforms to the ethics guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the Research Ethics Committee of University of Toyama, Japan.

**Clinical Diagnostic Criteria and Cardiac Evaluation**

LVNC diagnosis was determined on echocardiography based on the following criteria: (1) the characteristic 2-layered appearance of the myocardium, with an increased ratio of non-compacted layer to compacted layer (N/C ratio) >2.0 at end-diastole and the disease process observed in >1 ventricular wall segment; and (2) multiple deep intertrabecular recesses communicating with the ventricular cavity, on color Doppler imaging.\(^1\)

Echocardiography videotapes were reviewed and interpreted by 2 independent pediatric cardiologists (K.H. and S.W.O.) to confirm the diagnosis of LVNC and measure the LV wall thicknesses. Echocardiographic data included left ventricular diastolic diameter (LVDD), LV ejection fraction (LVEF), and the distribution and depth of prominent trabeculations in the LV. LVEF was calculated using the Pombo single-plane method. LVDD as defined by the actual tissue-blood interface, was measured at the level of the LV minor axis, approximately at the mitral valve leaflet tips on 2-D imaging, with the tip of the non-compacted portion chosen according to the American Society of Echocardiography Committee recommendations.\(^2\)

N/C ratio was measured in 5 wall segments of the LV at end-diastole: 4 wall segments of the anterior, lateral, posterior walls, and the interventricular septum at the level of the papillary muscles in short-axis view; and 1 wall segment at the apex in long-axis view. The final N/C ratio in each wall segment was the average of 3 measurements. We measured N/C ratio at the level of papillary muscles and at the apex. We calculated N/C ratio and assessed and summarized non-compaction scores from 5 wall segments. The thickness of the LV posterior wall (LVPW), the thickness of the compacted layer in the LV posterior wall (LVPWC), and the LVDD were expressed as Z-scores based on body surface area.\(^21\)\(^22\) We evaluated the correlation between different echocardiographic parameters and LVEF.

For the purposes of this analysis, DCM phenotype LVNC was echocardiographically defined as a combination of abnormal LV function (LVEF Z-score ≤−2) and LV dilation (LV end-diastolic dimension [LVEDD] Z-score >2) or the presence of at least moderately depressed LV function, defined as LVEF Z-score ≤−3 regardless of LV size.\(^23\)\(^24\)\(^25\) All patients in this study had DCM phenotype LVNC were included in this study.

**Mutation Screening**

Blood was obtained from patients and their relatives after informed consent, and genomic DNA was extracted from whole blood using QuickGene DNA whole blood kit S (KURABO, Osaka, Japan). NGS of a total of 73 cardiac disorder-related genes associated with cardiomyopathies and channelopathies (Table S1) was performed using an IonPGM system (Life Technologies, Carlsbad, CA, USA). This custom panel utilized 2 separate polymerase chain reaction (PCR) primer pools, yielding a total of 1,870 amplicons that were used to generate target amplicon libraries. Genomic DNA samples were PCR amplified using the custom panel and an Ion AmpliSeq Library Kit v2.0 (Life Technologies). Individual samples were labeled using an Ion Xpress Barcode Adapters Kit (Life Technologies) and then pooled at equimolar concentrations. Emulsion PCR and ion sphere particle (ISP) enrichment were performed using the Ion PGM Template Hi-Q OT2 200 Kit (Life Technologies), according to the manufacturer’s instructions. ISP were loaded onto a 316 chip and sequenced using an Ion PGM Hi-Q Sequencing 200 Kit (Life Technologies).

**Sanger Sequencing**

For all candidate pathogenic variants that passed these selection criteria, Sanger sequencing was used to validate the NGS results. For this, the nucleotide sequences of amplified fragments were analyzed on direct sequencing in both directions using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and sequence analysis was performed using an ABI 3130xl automated sequencer (Applied Biosystems).

**Data Analysis and Variant Classification**

Torrent Suite and Ion Reporter Software 5.0 (Life Technologies) were used to perform primary, secondary, and tertiary analyses, including optimized signal processing, base calling, sequence alignment, and variant analysis. The
The allelic frequency of all detected variants was determined using the Exome Aggregation Consortium (ExAC) database and the Human Genetic Variation Database (HGVD), which contain data for 1,208 Japanese individuals. All variants with a minor allele frequency (MAF) ≥0.05% in the ExAC and HGVD population were filtered out. We obtained functional or/and segregation analysis data on previously reported variants from both the human genome mutation database (HGMD) and the ClinVar disease mutation database. To evaluate the pathogenicity of the...
remaining variants, we utilized 7 different in silico predictive algorithms: FATHMM, SIFT, PROVEAN, Align GVGD, MutationTaster2, PolyPhen2, and CADD (Table S2). Variants predicted to be deleterious/pathogenic by ≥5 of the 7 in silico algorithms were considered likely pathogenic.

Frequency of Rare Variants in Control Population Data
Differences in proportions of rare variants vs. controls from the ExAC (east Asian) and HGVD databases were assessed using Fisher’s exact test, with P<0.05 considered statistically significant. Potential pathogenicity of the variants was evaluated based on allele frequency, as recommended by recent guidelines for interpreting sequence variants.26

Statistical Analysis
Continuous variables are expressed as mean±SD. Categorical variables are shown as count and percentage. Continuous variables were compared using unpaired t-test, non-parametric Mann-Whitney test, or 1-way analysis of variance. Categorical variables were compared using chi-squared statistics or Fisher’s exact test, as appropriate. Time-to-event data are presented as Kaplan-Meier estimates and were compared using log-rank test. Baseline variables that were considered clinically relevant or that had a significant univariate relationship with an adverse event were entered into the multivariable Cox proportional hazards regression models. Variables for inclusion were carefully chosen, given the number of events, to ensure parsimony in the final models. Statistical analysis was performed using JMP version 13 (SAS institute, Cary, NC, USA). P<0.05 was considered significant.

Results

Demographics and Clinical and Cardiological Characteristics
A total of 92 Japanese probands with DCM phenotype LVNC (male, n=56; female, n=36; median age, 1.4 months; range, 0–168 months) were enrolled (Table 1), and 15 patients with TAZ variants (median age, 1.0 months; range, 0–144 months) were identified in this cohort. All 15 patients were male and had a high likelihood of a family history of cardiomyopathy (Figure 1). Most presented with symptoms of chronic heart failure, such as poor sucking, growth failure, tachypnea, or dyspnea. None was born preterm (i.e., <36 weeks of gestation) and no intrauterine growth retardation was reported, including the patient who was diagnosed as a fetus. Growth retardation, myopathic face, decreased myodynamia, and decreased deep tendon reflex were not noted at the time of diagnosis but became apparent in 4 patients after diagnosis. No patient had severe mental retardation. Neutropenia was observed intermittently in 9 patients during the follow-up period. Seven patients were identified as having 3-methylglutaconic aciduria (3-MGCA) at diagnosis.

Fourteen patients were hospitalized at least once for chronic heart failure episodes but none of them had a history of thrombosis. Echocardiography showed more prominent trabeculations in the LV in the patients with TAZ variants than in the TAZ-negative patients (Figure 2). Average LVEF was 32.0±15.9% (Table 1). Twelve patients
had normal sinus rhythm, and 3 had ventricular tachycardia following the diagnosis of LVNC. Four patients died during follow-up, and 2 underwent heart transplantation.

**Molecular Analysis**

Ten different TAZ variants were detected in the 15 subjects (Tables 2, S3), consisting of 1 frameshift, 6 missense, and 3 splicing defect mutations. Of these, 3 missense substitutions were novel (not previously reported): c.476A>C, p.Gln159Pro; c.505C>T, p.Leu169Phe; c.646G>A, p.Gly216Arg. The evolutionary conservation of the mutated amino acids is shown in Figure S1. Some of these variants have been previously reported in patients with LVNC,9–27 but none has been identified in East Asian controls in ExAC. The OR for the association between the variant and the risk of disease were all significantly >1.0, and Fisher’s exact P-values were all <0.05.

**Genotype-Phenotype Correlation in TAZ**

Comparisons of physical findings and outcomes between patients positive or negative for TAZ variants are given in Table 3. The onset of disease in the TAZ-positive group was significantly earlier than in the other patients (92.3% vs. 62.3%, P=0.0182). The TAZ-positive group were more commonly male (100% vs. 53.2%, P=0.0003), and had a higher frequency of positive family history (73.3% vs. 20.8%, P=0.0001) than the other patients. On echocardiography, all had high N/C ratio in the posterior wall, lateral wall, and apex, and tended to have thick LV septal wall. The TAZ-positive group had higher thickness z-scores for the LVPW (8.55±2.60 vs. 5.81±2.56, P=0.0103), and the LVPWC (−2.55±1.55 vs. −5.34±2.65, P=0.0091) than the other patients (Figure 3). This suggests that LVNC patients with TAZ variants tend to have chronic heart failure during early infancy, and thick LVPW.

**Adverse Events: Patient Characteristics**

The number of composite events including death or heart transplantation was not significantly higher in the TAZ-positive group compared with the other LVNC patients, although a high occurrence of cardiac events was seen (P=0.7792; Figure 4). On multivariable proportional hazards modeling, male sex and positive family history were independent predictor for TAZ variants (Table 4). The area under the curve was 0.83 and 0.79 for LVPWC and LVPW z-score to predict TAZ variants, respectively (Figure S2).

**Discussion**

This is the first large retrospective study of the effect of TAZ variants on phenotype and outcomes in DCM phenotype LVNC patients. In this study, we identified 3 features: (1) TAZ variants are commonly identified in DCM phenotype LVNC patients (15/92, 16.3%), especially in male patients; (2) most of the patients in the TAZ-positive group were diagnosed in the first 4 months of life, and had a higher frequency of positive family history; and (3) the TAZ-positive group tended to have a thick non-compacted layer in the LV and a thick LV wall.

We identified 3 novel non-synonymous variants in TAZ in 5 patients with LVNC at highly conserved loci.30 Tafazzin is a phospholipid transacylase, which is located in the inner leaflet of the mitochondrial membrane and plays an important role in cardiolipin remodeling.31,32 TAZ...
### Table 2. Frequency of Rare TAZ Variants in the Control Population Databases

| Family / ID | cDNA change | Protein change | db SNP | ExAC % (All individuals) | HGVD % |
|-------------|-------------|----------------|--------|--------------------------|--------|
| TAZ-2       |             |                |        |                          |        |
| III-1,2     | c.109+1G>C  |                | –      | –                        | –      |
| TAZ-5       |             |                |        |                          |        |
| III-1       | c.109+6T>G  |                | –      | –                        | –      |
| Sporadic 1  |             |                |        |                          |        |
| II-1        | c.157dupC   | p.Leu53Profs*81| –      | –                        | –      |
| TAZ-6       |             |                |        |                          |        |
| III-1,2     | c.476A>C†   | p.Gln159Pro    | –      | –                        | –      |
| TAZ-4       |             |                |        |                          |        |
| III-3       | c.505C>T†   | p.Leu169Phe    | –      | –                        | –      |
| Sporadic 2  |             |                |        |                          |        |
| II-1        | c.526C>T    | p.His176Tyr    | –      | –                        | –      |
| TAZ-3       |             |                |        |                          |        |
| III-4       | c.553A>G    | p.Met185Val    | –      | –                        | –      |
| Sporadic 3  |             |                |        |                          |        |
| II-1        | c.589G>A    | p.Gly197Arg    | –      | –                        | –      |
| TAZ-7       |             |                |        |                          |        |
| III-5       | c.646G>A†   | p.Gly216Arg    | –      | –                        | –      |
| TAZ-1       |             |                |        |                          |        |
| VI-1,2      | c.647–1G>C  |                | –      | –                        |        |
| V-10        |             |                |        |                          | –      |

Table 2. Frequency of Rare TAZ Variants in the Control Population Databases

| Family / ID | Genotype | ExAC (East Asian) | Risk | Frequency 95% CI | Fisher’s exact P-value | Classification |
|-------------|----------|-------------------|------|-------------------|------------------------|----------------|
| TAZ-2       |          |                    |      |                   |                        |                |
| III-1,2     | 2        | 0                  | 89.97| 4.30–1,880.61     | 0.0028                 | Pathogenic      |
| TAZ-5       |          |                    |      |                   |                        |                |
| III-1       | 1        | 0                  | 53.76| 2.18–1,324.13     | 0.0530                 | Likely pathogenic|
| Sporadic 1  |          |                    |      |                   |                        |                |
| II-1        | 1        | 0                  | 53.76| 2.18–1,324.13     | 0.0530                 | Pathogenic      |
| TAZ-6       |          |                    |      |                   |                        |                |
| III-1,2     | 2        | 0                  | 89.97| 4.30–1,880.61     | 0.0028                 | Pathogenic      |
| TAZ-4       |          |                    |      |                   |                        |                |
| III-3       | 1        | 0                  | 53.76| 2.18–1,324.13     | 0.0530                 | Likely pathogenic|
| Sporadic 2  |          |                    |      |                   |                        |                |
| II-1        | 1        | 0                  | 53.76| 2.18–1,324.13     | 0.0530                 | Likely pathogenic|
| TAZ-3       |          |                    |      |                   |                        |                |
| III-4       | 1        | 0                  | 53.76| 2.18–1,324.13     | 0.0530                 | Likely pathogenic|
| Sporadic 3  |          |                    |      |                   |                        |                |
| II-1        | 1        | 0                  | 53.76| 2.18–1,324.13     | 0.0530                 | Likely pathogenic|
| Sporadic 4  |          |                    |      |                   |                        |                |
| II-1        | 1        | 0                  | 53.76| 2.18–1,324.13     | 0.0530                 | Likely pathogenic|
| TAZ-7       |          |                    |      |                   |                        |                |
| III-5       | 1        | 0                  | 53.76| 2.18–1,324.13     | 0.0530                 | Likely pathogenic|
| TAZ-1       |          |                    |      |                   |                        |                |
| VI-1,2      | 3        | 0                  | 126.48| 6.51–2,457.45    | 0.0001                 | Pathogenic      |
| V-10        |          |                    |      |                   |                        |                |

*Novel variant, db SNP, single nucleotide polymorphism database; ExAC, Exome Aggregation Consortium; HGVD, Human Genetic Variation Database; TAZ, tafazzin.
### Table 3. DCM Phenotype LVNC Patient Characteristics vs. TAZ Variant Status

| Variables                      | All patients (n=92) | TAZ (n=15) | Others (n=77) | P-value |
|--------------------------------|---------------------|------------|---------------|---------|
| Age at diagnosis (months)      | 1.37 (0–168)        | 1.00 (0–144) | 1.73 (0–168) | 0.7789  |
| Age at diagnosis <4 months     | 62 (66.7)           | 14 (92.3)  | 48 (62.3)     | 0.0182  |
| Male                           | 56 (60.0)           | 15 (100)   | 41 (53.2)     | 0.0003  |
| Family history of CM           | 26 (27.8)           | 11 (73.3)  | 16 (20.8)     | 0.0001  |
| Symptoms at diagnosis          |                     |            |               |         |
| Asymptomatic                   | 22 (22.2)           | 5 (33.3)   | 17 (22.1)     | 0.3410  |
| CHF                            | 65 (72.2)           | 10 (66.7)  | 55 (71.4)     | 0.4641  |
| Arrhythmia                     | 2 (2.2)             | 0 (0)      | 2 (2.6)       | 1.0000  |
| Clinical presentation          |                     |            |               |         |
| CHF                            | 78 (86.7)           | 11 (84.6)  | 67 (87.0)     | 0.2171  |
| Systemic embolic events        | 4 (4.4)             | 0 (0)      | 4 (5.2)       | 1.0000  |
| Arrhythmia                     | 19 (21.1)           | 3 (23.1)   | 16 (20.8)     | 1.0000  |
| VT                             | 7 (7.8)             | 3 (23.1)   | 4 (5.2)       | 0.0827  |
| SVT                            | 8 (8.9)             | 1 (7.7)    | 7 (9.1)       | 1.0000  |
| CAVB                           | 1 (1.1)             | 0 (0)      | 1 (1.3)       | 1.0000  |
| Extracardiac complication      |                     |            |               |         |
| HF requiring hospitalization   | 81 (90.2)           | 14 (93.3)  | 69 (89.6)     | 0.3787  |
| Event (HTx)                    | 4 (4.4)             | 2 (13.3)   | 3 (3.9)       | 0.1855  |
| Event (death)                  | 23 (25.6)           | 4 (30.8)   | 19 (24.7)     | 1.0000  |
| Echocardiography               |                     |            |               |         |
| LVEF (%)                       | 32.96±13.42         | 32.01±15.93| 32.88±12.77   | 0.8182  |
| LVDD Z-score                   | 4.73±2.79           | 4.47±2.69  | 4.77±2.83     | 0.7418  |
| N/C ratio                      |                     |            |               |         |
| Anterior wall                  | 0.77±0.62           | 1.11±1.04  | 0.74±0.55     | 0.1432  |
| Posterior wall                 | 4.74±2.49           | 4.56±2.29  | 4.76±2.54     | 0.8426  |
| Lateral wall                   | 2.79±1.04           | 2.53±1.09  | 2.82±1.02     | 0.4859  |
| Septal wall                    | 0.71±0.75           | 0.49±0.27  | 0.74±0.77     | 0.3853  |
| Apex                           | 4.44±2.40           | 4.15±1.95  | 4.49±2.47     | 0.7240  |
| Mean of 5 segments             | 2.70±0.90           | 2.57±0.94  | 2.72±0.90     | 0.6834  |
| LVPW thickness Z-score         | 6.13±2.69           | 8.55±2.60  | 5.81±2.56     | 0.0103  |
| LVSW thickness Z-score         | 2.70±2.69           | 4.76±2.71  | 2.43±3.40     | 0.0885  |
| LVPWC thickness Z-score        | −5.01±2.69          | −2.55±1.55 | −5.34±2.65    | 0.0091  |

Data given as mean±SD, n (%) or median (range). CAVB, complete atrial ventricular block; CHF, chronic heart failure; LVDD, left ventricular diastolic diameter; LVPW, left ventricular posterior wall; LVPWC, compacted layer in left ventricular posterior wall; LVSW, left ventricular septal wall; N/C, ratio of non-compacted layer to compacted layer; SVT, supraventricular tachycardia. Other abbreviations as in Table 1.

![Figure 3](image_url)

**Figure 3.** (A) Left ventricular posterior wall (LVPW) and (B) compacted layer in the left ventricular posterior wall (LVPWC) thickness z-score in patients with dilated cardiomyopathy phenotype left ventricular non-compaction according to tafazzin (TAZ) variant status.
variants may change the mitochondrial function and cause dysfunction in many organs. Diagnosis of BTHS is difficult because neonatal or infantile patients are diagnosed with heart failure while the commonly associated phenotypes, neutropenia and skeletal myopathy, are rarely seen in this age group. This study shows that molecular analysis of the TAZ gene is a powerful tool for the diagnosis of BTHS. Moreover, of these variants, 1 was newly identified using NGS but had been previously missed on direct DNA sequencing (sporadic 2) even though the patient had BTHS manifestations, including heart failure, neutropenia, 3-MGCA, and motor delay. A false-negative result may be avoided by genetic testing with NGS instead of conventional direct DNA sequencing.

Most of these patients were diagnosed in the first 4 months of life, and the disease onset in the TAZ-positive group was significantly earlier than in the other DCM phenotype LVNC patients. There was no significance difference in mortality between the TAZ-positive group and the other patients. In recent retrospective studies of children with DCM phenotype LVNC, the event of death or transplantation occurred significantly more often than in those with other phenotypes.18,33

Interestingly, the TAZ mutation carriers had a thicker non-compacted layer in the LV, and had a higher thickness z-score for the LVPW and for the LVPWC than the other patients. The TAZ-positive group had more prominent trabeculations in the LV, resulting in thick LV walls, supporting previous studies showing that TAZ mutation carriers have prominent trabeculations and thick LV walls.34,35 TAZ deficiency may disturb mitochondrial function, leading to abnormalities of energy production and utilization. Ultrastructural abnormalities observed in patients with neuromuscular disorders, including BTHS, are generally non-specific and include increased numbers of mitochondria, abnormally shaped mitochondria, distorted cristae, sarcomeric derangement, immature cardiomyocytes, lipid-like inclusions, enlarged interstitial spaces, increased interstitial collagen, or increased glycogen.36 Wang et al used induced pluripotent stem cell-derived cardiomyocytes and found that TAZ deficiency in Barth syndrome impairs sarcomere assembly and contractile stress generation.37 As a result, TAZ deficiency may lead to sarcomeric dysfunction because the sarcomere needs ATP, which may cause the differing morphological features from other LVNC patients. This suggests that the degree of LVPW trabeculation and wall thickness is a good predictor for identifying the patients with variants in TAZ. Thus greater attention should be

| Table 4. Independent Predictors of TAZ Variants in Pediatric DCM Phenotype LVNC |
|-------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| | Variables                 | Univariable analysis | Multivariable analysis | | | | |
| | | OR 95% CI | P-value | OR 95% CI | P-value | | | |
| | Age at diagnosis <4 months | 8.458 1.571–157.218 | 0.009 | 5.591 0.829–112.455 | 0.0805 | | | |
| | Male | 29,425,520 9.006×10^21– | <0.0001 | 28,712,888 6.502– | <0.0001 | | | |
| | FaH of CM | 10.484 2.944–37.329 | 0.0003 | 7.981 2.090–34.661 | 0.0021 | | | |
| | LVPWC thickness Z-score >−4.5 | 8.454 1.317–165.958 | 0.0022 | | | | |
| | LVPW thickness Z-score >8.4 | 14.062 2.567–111.180 | 0.0022 | | | | |

Abbreviations as in Tables 1,3.
paid to male patients with DCM phenotype LVNC because they are more likely to develop heart failure during early infancy and have prominent trabeculations in the LV. Therefore, the identification of patients with TAZ variants who have only heart failure may lead to management with precision medicines specific for mitochondrial cardiomyopathies, such as BTHS.

**Study Limitations**

The retrospective design and the considerable selection bias were the major limitations of this study. It is also important to note that the present study had selection bias because the presence of LVNC was defined before the patients were enrolled. Expanded genetic studies with careful phenotypic and familial investigations using NGS will help classify genetic variants in the future, and further functional studies, including analysis of nonsense mediated decay, are warranted to identify the underlying molecular mechanisms of LVNC.

**Conclusions**

The patients with TAZ variants had a higher frequency of early onset of disease, positive family history, and higher LVPW thickness Z-score than in those without TAZ variants, while the long-term prognosis and mortality of both groups were similar. This study provides new insight into the impact of DCM phenotype LVNC and suggests there may be clinical advantages available for LVNC patients with TAZ variants, given that tailored therapeutic interventions may become available. Therefore, identifying patients who should be observed with greater caution during the early stage of the disease remains a critical issue. Further studies are needed to clarify the potential benefits for LVNC patients with TAZ variants.

**Disclosures**

The authors declare no conflict of interest.

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Supplementary Files

Supplementary File 1
Figure S1. Homology of amino acid sequences of sarcomere genes around sequence variations.
Figure S2. Receiver operating characteristics (ROC) curves for left ventricular posterior wall (LVPW) and compacted layer in the left ventricular posterior wall (LVPWC) thickness z-scores to discriminate between the tafazzin (TAZ) group vs. other patients.
Table S1. Analyzed genes associated with inherited cardiac disease
Table S2. In silico predictive algorithms used in this study
Table S3. Novel TAZ mutations, absent in ExAC and HGVD
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