ORIGINAL RESEARCH

A Wide and Specific Spectrum of Genetic Variants and Genotype–Phenotype Correlations Revealed by Next-Generation Sequencing in Patients with Left Ventricular Noncompaction

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***Background*—**Left ventricular noncompaction (LVNC) has since been classified as a primary genetic cardiomyopathy, but the genetic basis is not fully evaluated. The aim of the present study was to identify the genetic spectrum using next-generation sequencing and to evaluate genotype–phenotype correlations in LVNC patients.

***Methods and Results*—**Using next-generation sequencing, we targeted and sequenced 73 genes related to cardiomyopathy in 102 unrelated LVNC patients. We identified 43 pathogenic variants in 16 genes in 39 patients (38%); 28 were novel variants. Sarcomere gene variants accounted for 63%, and variants in genes associated with channelopathies accounted for 12%. *MYH7* and *TAZ* pathogenic variants were the most common, and rare variant collapsing analysis showed variants in these genes contributed to the risk of LVNC, although patients carrying *MYH7* and *TAZ* pathogenic variants displayed different phenotypes. Patients with pathogenic variants had early age of onset and more severely decreased left ventricular ejection fractions. Survival analysis showed poorer prognosis in patients with pathogenic variants, especially those with multiple variants: All died before their first birthdays. Adverse events were noted in 17 patients, including 13 deaths, 3 heart transplants, and 1 implantable cardioverter-defibrillator insertion. Congestive heart failure at diagnosis and pathogenic variants were independent risk factors for these adverse events.

***Conclusions*—**Next-generation sequencing revealed a wide spectrum of genetic variations and a high incidence of pathogenic variants in LVNC patients. These pathogenic variants were independent risk factors for adverse events. Patients harboring pathogenic variants showed poor prognosis and should be followed closely. **(*J Am Heart Assoc*. 2017;6:e006210. DOI: 10.1161/JAHA.117.006210.)**

Key Words: genetics • noncompaction cardiomyopathy • prognosis

Left ventricular noncompaction (LVNC) was originally described as cross-linked infantile cardiomyopathy with poor prognosis[1] but has since been classified as a primary genetic cardiomyopathy by the American Heart Association.[2] LVNC is characterized by a pattern of prominent trabecular meshwork and deep intertrabecular recesses communicating with the left ventricular cavity. LVNC is postulated to be caused by an arrest of the normal process of intrauterine endomyocardial morphogenesis. LVNC may be a distinct disorder but also may be associated with other cardiomyopathies.[2–7] With the development of sequencing technologies, multiple gene variants have been found related to LVNC, but the genetics of LVNC have not been fully evaluated. Previous studies have shown that sarcomere gene variants likely play an important role in patients

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Accompanying Appendix S1 and Tables S1 through S3 are available at http://jaha.ahajournals.org/content/6/9/e006210/DC1/embed/inline-supplementary-material-1.pdf

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Clinical Perspective

What Is New?

- This research revealed a wide spectrum of genetic variants and high incidence of novel pathogenic variants using a focused next-generation sequencing strategy in a cohort of 102 patients with left ventricular noncompaction.

What Are the Clinical Implications?

- The presence of a pathogenic variant was an independent risk factor for death, heart transplantation, or implantable cardioverter-defibrillator insertion in patients with left ventricular noncompaction, and the prognosis was even worse in patients with double pathogenic variants or *TAZ* variants.

with LVNC[8] but do not predict clinical phenotype.[9] Next-generation sequencing (NGS) was used recently because of the ability to investigate multiple genes at reasonable cost. The aim of this study was to investigate the genetic landscape of LVNC and to identify genotype–phenotype correlations in the largest cohort of well-phenotyped Japanese LVNC patients.

Methods

Clinical Evaluation

Unrelated childhood patients were recruited from 2001 to 2016 from 61 Japanese hospitals with divisions of pediatric cardiology. A total of 102 patients with LVNC were included in this study. Three patients had Barth syndrome; none had neuromuscular disorders. In addition, patients with congenital heart disease that induced significant hemodynamic changes or with insufficient clinical information were excluded. Clinical evaluation consisted of clinical presentation and symptoms; a personal and family history (patient's biological family members showed existence of any cardiomyopathy disease, not only LVNC but also other cardiomyopathy or family members [parents or brother sisters]), arrhythmia, thromboembolism, ECG, 2-dimensional Doppler, and color Doppler echocardiography. The diagnosis of heart failure was based on clinical symptoms of feeding difficulty, tachypnea, and cyanosis and findings of decreased left ventricular ejection fraction (LVEF) in the left ventricle on echocardiography and cardiomegaly on chest x-ray. A diagnosis of LVNC was made according to (1) the characteristic 2-layered appearance of the myocardium, with an increased N/C ratio (N/C>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, as demonstrated by color Doppler imaging.[3]

Informed consent was obtained from all patients' parents, 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.

Mutation Screening

Genomic DNA was extracted from whole blood using a QuickGene DNA whole blood kit S (Kurabo). NGS of 73 cardiac disorder–related genes associated with cardiomyopathies and channelopathies (Table S1) was performed using an IonPGM system (Life Technologies). This custom panel utilized 2 separate polymerase chain reaction primer pools, yielding a total of 1870 amplicons and used to generate target amplicon libraries. Genomic DNA samples were polymerase chain reaction–amplified using the custom panel and an Ion AmpliSeq Library Kit v2.0 (Life Technologies, Carlsbad, CA). Individual samples were labeled using an Ion Xpress Barcode Adapters Kit (Life Technologies) and then pooled at equimolar concentrations. Emulsion polymerase chain reaction and ion sphere particle enrichment were performed using the Ion PGM HiQ OT2 Kit (Life Technologies), according to the manufacturer's instructions. Ion sphere particles were loaded onto a 316 chip and sequenced using an Ion PGM HiQ Sequencing Kit (Life Technologies).

Data Analysis and Variant Classification

Torrent Suite and Ion Reporter software version 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 allelic frequency of all detected variants was determined using the Exome Aggregation Consortium (ExAC) East Asian database and the Human Genetic Variation Database (HGVD), which contains data for 1208 Japanese persons.[10] Rare variants such as those single-nucleotide polymorphisms with a minor allele frequency (MAF) below some threshold in the combined set of cases and controls were selected.[11] All variants with a MAF ≥0.05% among the ExAC East Asian and HGVD populations were filtered out.[12,13] We utilized 7 different in silico predictive algorithms to improve the accuracy of evaluating the pathogenicity of the remaining variants: FATHMM, SIFT, PROVEAN, Align GVGD, MutationTaster2, PolyPhen2, and CADD (URLs listed in Table S2). Variants predicted to be deleterious or pathogenic by at least 5 of the 7 in silico algorithms were considered likely pathogenic. The pathogenicity of the detected variant was based on the guidelines of the American College of Medical Genetics and Genomics.[13]

Sanger Sequencing

For all candidate pathogenic variants that passed these selection criteria, Sanger sequencing was used to validate the

NGS results. The nucleotide sequences of amplified fragments were analyzed by direct sequencing in both directions using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), and sequence analysis was performed using an ABI 3130xl automated sequencer (Applied Biosystems).

Assessment of the Frequency of Rare Variants in Control Population Data

Differences in proportions of rare variants versus controls from the ExAC East Asian and HGVD data were assessed using the Fisher 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.[13]

Gene-Based Collapsing Test

We used a genic collapsing test to confer risk genes of LVNC.[14,15] Each gene was indicated as carrying or not carrying a "qualifying" variant. A *qualifying variant* was defined as a variant with an MAF cutoff of <0.05% among the ExAC East Asian population. *Qualified variants* were defined as nonsynonymous, frameshift, and splice-site variants.

Statistical Analysis

Statistical analysis was performed with SPSS (version 24; IBM Corp) software and R software. The unpaired *t* test or the χ^2 test was used to compare variables. $P<0.05$ was considered statistically significant. Important prognostic factors were used in the univariate analysis and then in Firth regression using R software.[16] The event-free rate for the combined end point of death, heart transplantation (HT), or implantable cardioverter-defibrillator (ICD) insertion was calculated by the Kaplan-Meier method and compared using the log-rank test. The Fisher exact test was performed for each gene in collapsing analysis with a nominal significance level $<1.37\times10^{-4}$ according to Bonferroni correction for the number of assessable genes.

Results

Baseline Clinical Characteristics

A total of 102 patients were enrolled in this study; 54 were male and 48 were female, with an age range from fetus to 12 years (mean age: 1.8±0.4 years; Table 1). Pathogenic variants were identified in 39 patients (38%) who presented with a much earlier age of onset and lower LVEF ($P<0.05$) than those without pathogenic variants. The majority (76.9%) of patients with pathogenic variants presented with congestive heart failure at diagnosis. We divided the LVNC patients into 2 types: those with systolic dysfunction (n=63) and those without systolic dysfunction (n=39). Pathogenic variants were more commonly detected in patients with systolic dysfunction (31/63, 49%) than in those without (9/39, 23%; $P=0.012$). Family history was more common in patients with pathogenic variants but did not reach statistical significance. Survival analysis showed that patients with pathogenic variants had worse prognosis than patients without; 26% of the patients with pathogenic variants died or underwent HT or ICD insertion (Figure 1).

Table 1. Characteristics of Patients With and Without Pathogenic Mutations

	P+ (n=39)	P− (n=63)	*P* Value
Sex, male:female	18:21	34:27	0.54
Age at onset, y	0.45±0.2	2.7±0.6	0.003
CHF at diagnosis, n (%)	30 (76.9)	32 (50.8)	0.01
Family history, n (%)	12 (30.8)	12 (19)	0.81
LVEF, %	37±2.0	46.3±3.0	0.01
LVDD *z* score	1.59±0.18	1.44±0.56	0.79

CHF indicates congestive heart failure; LVEF, left ventricular ejection fraction; LVDD, left ventricular end-diastolic dimension; P+, patients with pathogenic mutations; P−, patients with no or nonpathogenic mutations.

Genetic analysis

NGS of samples from the 102 patients yielded 540 830±11 986 sequence reads per person. The mean

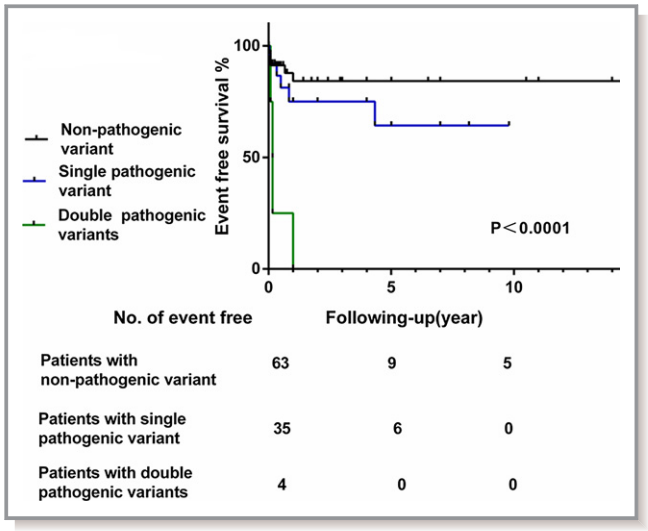

Figure 1. Event-free survival to the combined end point of death, heart transplantation, and implantable cardioverter-defibrillator insertion of patients with double pathogenic, pathogenic, and nonpathogenic mutations.

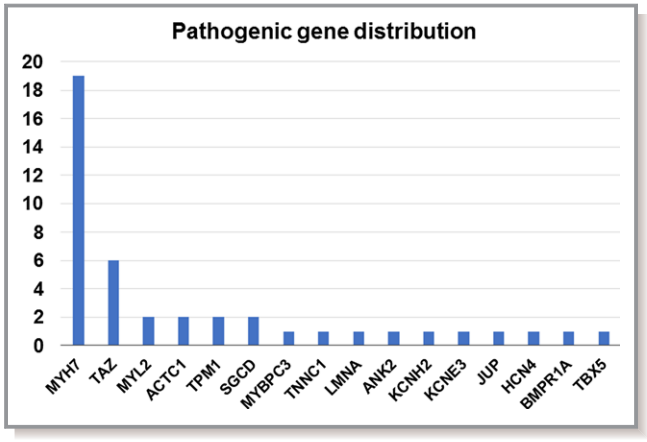

Figure 2. Pathogenic gene distribution of left ventricular noncompaction. The number of pathogenic mutations identified in each gene in which at least 1 mutation was identified.

read length per sample was 163.6±1.1 base pairs, and the mean depth of base coverage was 247.0±5.8 reads; 95.23% had >10-fold coverage, and 92.5% had >20-fold coverage.

The distribution of pathogenic variants is shown in Figure 2. There were 43 pathogenic variants: 39 missense, 1 deletion, 1 nonsense, and 2 splice site variants. Sarcomere gene variants accounted for 63%, and variants in genes associated with channelopathies accounted for 12%. Overall, *MYH7* was most commonly mutated (n=19, 44%), followed by *TAZ* (n=6, 14%). There was only 1 pathogenic variant in each of the following genes: *MYBPC3, TNNC1, LMNA, ANK2, KCNH2, KCNE3, JUP, HCN4, BMPR1A,* and *TBX5*. Notably, this is the first report of pathogenic variants in *BMPR1A*, *ANK2*, and *TBX5* in LVNC patients. Ten missense variants were identified in *MYBPC3*, but 9 of them were filtered out because of their frequent occurrence (MAF >0.5%) in the ExAC East Asian or HGVD (Japanese) populations. Consequently, there is a significant difference in the prevalence of variants in *MYH7* and *MYBPC3* in this study, unlike other forms of cardiomyopathy (Table S3).

Twenty-nine novel variants (not detected in 60 706 persons of any race/ethnicity in the ExAC and HGVD databases) were identified in 12 genes: 19 novel variants in sarcomere genes (66%), including 12 *MYH7* variants, and 4 novel variants in *TAZ*. Novel pathogenic variants were also identified in *BMPR1A, HCN4, LMNA, SGCD,* and *TBX5* (Table S4).

In addition, 14 rare variants with MAF<0.05% in the 2 reference databases were identified in 7 genes (*ANK2, JUP, KCNE3, KCNH2, MYH7*, *MYL2* and *TAZ*; Table 2). None of them had been reported previously in East Asian controls in ExAC or HGVD. The odds ratios for the association between the variant and the risk of disease were all significantly >1.0, and the Fisher exact *P* values were all <0.05 (Table 2). The genic collapsing test revealed that *MYH7* (*P*=1.29E-17, ranked first) and *TAZ* (*P*=3.48E-9, ranked second) reached significance (adjusted a or $P<1.37\times10^{-4}$), strongly suggesting that variants in these genes contribute to an increased risk of LVNC. All other genes, including *MYBPC3, ANK2, TPM1* and *ACTC1*, did not reach the adjusted α (Table S5).

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

Gene	Variant	dbSNP	ExAC (All Individuals), %	HGVD, %	Genotype, Case (n=102)	ExAC (East Asian, n=4327)	Risk, OR	Frequency, 95% CI	*P* Value	Classification
ANK2	R321W	rs753032598	0.0025	…	1	0	127.9	1.08 to + ∞	0.0230	Likely pathogenic
JUP	E146K	rs146581757	0.002	…	1	0	127.9	1.08 to + ∞	0.0230	Likely pathogenic
KCNE3	R99H	rs121908441	0.0086	…	1	0	127.9	1.08 to + ∞	0.0230	Pathogenic
KCNH2	A561T	rs199472921	…	…	1	0	127.9	1.08 to + ∞	0.0230	Pathogenic
MYH7	R23W	rs730880828	0.0025	…	1	0	127.9	1.08 to + ∞	0.0230	Likely pathogenic
	L620P	rs199862338	…	…	1	0	127.9	1.08 to + ∞	0.0230	Likely pathogenic
	P838L	rs397516153	…	…	1	0	127.9	1.08 to + ∞	0.0230	Likely pathogenic
	R904C	rs727503253	0.00082	…	1	0	127.9	1.08 to + ∞	0.0230	Likely pathogenic
	E1801K	rs397516248	…	…	2	0	215.3	8.0 to + ∞	0.0005	Likely pathogenic
	E1914K	rs397516254	…	…	1	0	127.9	1.08 to + ∞	0.0230	Likely pathogenic
MYL2	P144fs	rs199567559	0.00082	…	1	0	127.9	1.08 to + ∞	0.0230	Likely pathogenic
TAZ	G197R	rs132630277	…	…	1	0	127.9	1.08 to + ∞	0.0230	Likely pathogenic
	c.109+1G>C	…	…	…	1	0	127.9	1.08 to + ∞	0.0230	Pathogenic

CI indicates confidence interval; ExAC, Exome Aggregation Consortium database; HGVD, Human Genetic Variation Database; OR, odds ratio.

Table 3. Characteristics of Patients With Single and Double Mutations

	Single Variant (n=35)	Double Variant (n=4)	*P* Value
Sex, male:female	15:20	3:1	0.32
Age of onset, y	0.5±0.2	0.001±0.001	0.43
CHF at diagnosis, n (%)	26 (74.3)	4 (100)	0.56
Family history, n (%)	12 (34.2)	0	0.29
LVEF, %	36.9±2.2	37.5±3.8	0.93
LVDD *z* score	1.51±0.19	2.31±0.34	0.19

Double heterozygous variants: *MYH7* and *JUP*, *MYH7* and *BMPR1A*, *TPM1* and *SGCD*, and *TAZ* and *KCNE3*. CHF indicates congestive heart failure; LVDD, left ventricular end-diastolic dimension; LVEF, left ventricular ejection fraction.

The Characteristics of Patients With Single or Double Pathogenic Variants

Double heterozygous variants were identified in 4 patients, all of whom presented with congestive heart failure during the fetal or neonatal periods and died before their first birthdays. Of note, none had family history of cardiomyopathy (Table 3). Survival analysis revealed that patients with double variants showed the worst prognosis compared with patients with a single variant and without variants (Figure 1). There were no differences in age of onset, heart failure at diagnosis, LVEF, and family history between the 2 groups (Table 3).

The characteristics of patients with adverse events

Adverse events were noted in 16 patients: 12 died, 3 underwent HT, and 1 underwent ICD insertion. Among those 16, double heterozygous variants were identified in 4 patients, and single variants were noted in 6, including variants in *TAZ* in 2. No pathogenic variants were identified in the remaining 6 patients (Table 4). The majority of patients with adverse events were boys (76%). All of these patients were diagnosed before their first birthday, except 1 who was diagnosed at age 4 years and underwent ICD insertion after 9 months of follow-up. Five patients were diagnosed during the fetal period, because of severe heart failure and hydrops fetalis, and died soon after birth. The multivariable proportional hazards model showed that congestive heart failure at diagnosis and pathogenic variant were independent risk factors for death, HT, or ICD insertion in all LVNC patients (Table 5).

Genotype–phenotype correlations

Variants found in participants with systolic dysfunction and details of for each participant are shown in Tables S6 and S7. Single sarcomere variants were identified in 24 patients, single nonsarcomere variants were found in 11, and double variants were noted in 4 patients (*MYH7* and *JUP*, *MYH7* and *BMPR1A*, *TPM1* and *SGCD*, *TAZ* and *KCNE3*; Table 4). There were no differences in age at onset, heart failure onset, LVEF,

Table 4. Characteristics of Patients With Adverse Events

ID	Gene and Variant	Age at Onset	Sex	Family History	CHF at Diagnosis	Outcome	Cause of Death
234	*SGCD* N99H; *TPM1* D14G	15 d	M	No	Yes	Death	CHF
274	*TAZ* H176Y; *KCNE3* R99H	Fetus	M	No	Yes	Death	CHF
280	*MYH7* K542N; *JUP* E146K	Fetus (30 WG)	M	No	Yes	Death	CHF
342	*MYH7* P838L; *BMPR1A* R284L	1 d	F	No	Yes	Death	CHF
159	*TAZ* splice donor c.109+1G>C	2 mo	M	Yes	Yes	Death	CHF
247	*MYH7* R712H	Fetus (32 WG)	F	No	Yes	HT	
312	*ACTC1* T231R	4 y	M	No	Yes	ICD insertion	
313	*TAZ* M185V	1 mo	M	Yes	Yes	HT	
233	*KCNH2* A561T	Fetus (25 WG)	M	No	Yes	Death	CHF
321	*TNNC1* E94A	4 mo	F	No	No	HT	
193	…	1 d	M	No	Yes	Death	CHF
275	…	1 d	M	No	Yes	Death	CHF
294	…	1 y	M	No	Yes	Death	CHF
356	…	15 d	M	Yes	Yes	Death	VF
367	…	Fetus	F	Yes	Yes	Death	CHF
416	…	1 mo	M	No	Yes	Death	CHF

CHF indicates congestive heart failure; F, female; HT, heart transplantation; ICD, implantable cardioverter-defibrillator; M, male; VF, ventricular fibrillation; WG, weeks of gestation.

Table 5. Multivariate Analysis of Risk Factors for LVNC

Variable	Univariable Survival Analysis		Multivariable Survival Analysis	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Age at onset, y	3.14 (1.17–8.42)	0.03	0.47 (0.12–2.61)	0.34
Family history	1.42 (0.46–4.43)	0.16	2.08 (0.65–5.97)	0.20
CHF at diagnosis	19.30 (2.98–20.31)	0.0003	46.24 (5.39–6097.7)	0.00002
Genotype positive	3.61 (1.27–10.20)	0.01	3.22 (1.12–11.22)	0.03

CHF indicates congestive heart failure; CI, confidence interval; HR, hazard ratio; LVNC, left ventricular noncompaction.

and family history between the sarcomere and nonsarcomere groups (Table 6). Survival analysis showed that the prognosis of patients with nonsarcomere variants was worse than that of patients with sarcomere variants (Figure 3).

Because *MYH7* and *TAZ* were predicted to significantly contribute to the risk of LVNC, we compared the characteristics of patients with variants in these genes (Table 7). The patients carrying *TAZ* variants displayed a distinct phenotype; all were male infants who presented with congestive heart failure and had worse prognoses. Three had Barth syndrome, 1 with double variants. Overall, 80% of the *TAZ* group had family history of cardiomyopathy; this was much higher than the *MYH7* group. The *TAZ* group presented with higher LVDD *z* scores and lower LVEF than the *MYH7* group. There were no differences in age at onset between the groups. In our study, we found that the clinical manifestation varied significantly in the patients with *MYH7* variants, from no symptoms to severe heart failure. Two patients with double variants of *MYH7* and another gene and 1 patient with *TAZ* and another variant were excluded from the analysis (Table 7). Survival analysis showed that the prognosis was significantly worse for patients with *TAZ* variants compared with patients with sarcomere gene variants (*P*=0.03; Figure 3).

Among the patients with nonsarcomere gene variants, 5 carried variants in channelopathy-related genes: *ANK2*, *KCNE3*, *KCNH2*, *HCN4*, and *JUP*. The ECG of the patient with the *KCNE3* variant showed left bundle-branch block. ECGs of the patients with *ANK2*, *HCN4* and *LMNA* variants showed normal or nonspecific changes. The patient with the *KCNH2* variant died at 2 weeks after birth due to severe congestive heart failure; however, no specific changes were identified on ECG.

One patient who carried both *MYH7* and *BMPR1A* variants was diagnosed during the fetal period and died after 1 year of follow-up. We extracted DNA from her postmortem heart and found the same variants in *MYH7* and *BMPR1A* that were detected previously in blood samples (Figure 4).

The variant in *TPM1* appeared de novo (Figure 5A), as neither parent nor a brother carried this variant.

A variant in *MYH7*, c.1085T>G (p. Met362Arg), was identified in a family with LVNC and Ebstein anomaly (Figure 5B); we previously reported this variant[14] using a candidate gene approach. However, no additional pathogenic variants, inherited from the unaffected mother, were identified in the offspring with Ebstein anomaly that could account for

Table 6. Characteristics of Patients With Sarcomere and Nonsarcomere Mutations

	Sarcomere Variant (n=24)	Nonsarcomere Variant (n=11)	*P* Value
Sex male:female	8:16	3:8	0.99
Age of onset, y	0.7±0.3	0.15±0.07	0.26
CHF at diagnosis, n (%)	15 (62.5)	10 (91)	0.12
Family history, n (%)	6 (34.8)	6 (54.5)	0.13
LVEF, %	39.4±2.3	31.8±4.7	0.11
LVDD *z* score	1.24±0.2	2.1±0.4	0.04

CHF indicates congestive heart failure; LVDD, left ventricular end-diastolic dimension; LVEF, left ventricular ejection fraction.

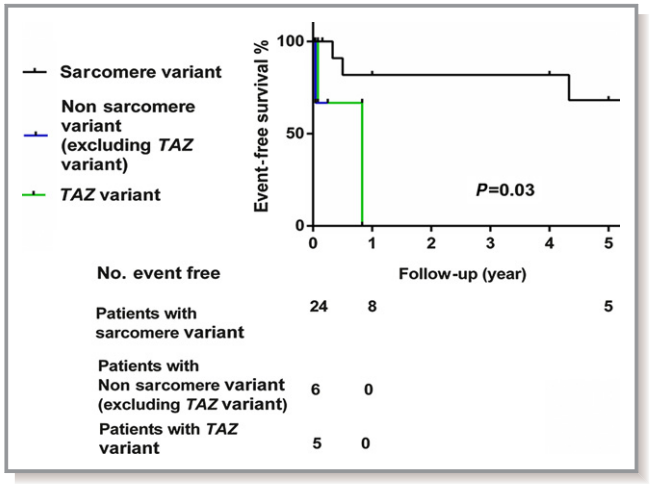

Figure 3. Event-free survival to the combined end point of death, heart transplantation, and implantable cardioverter-defibrillator insertion of patients with sarcomere, nonsarcomere (excluding *TAZ* mutations), or *TAZ* mutations.

Table 7. Characteristics of Patients With *MYH7* or *TAZ* Mutations

	MYH7 (N=17)	*TAZ* (N=5)	*P* Value
Sex, male:female	5:12	5:0	0.01
Age at onset, y	0.5±0.4	0.3±0.1	0.71
CHF at diagnosis, n (%)	10 (58.8)	5 (100)	0.13
Family history, n (%)	4 (23.5)	4 (80)	0.039
LVEF, %	39.8±3.2	20.4±5.6	0.008
LVDD *z* score	1.07±0.27	3.13±0.36	0.001

Three patients with double mutation of *MYH7* and another gene and 1 patient with *TAZ* and another mutation were excluded. CHF indicates congestive heart failure; LVDD, left ventricular end-diastolic dimension; LVEF, left ventricular ejection fraction.

the phenotypic difference between the father and the children.

Discussion

In summary, use of a focused NGS strategy in a large cohort of 102 LVNC patients revealed a wide and specific spectrum of genetic variations and a high incidence of novel pathogenic variants in LVNC patients. In addition, we found poorer prognosis in the patients with pathogenic variants, and the detection of a pathogenic variant was an independent risk factor for death, HT, and ICD insertion.

There appears to be a distinct spectrum of gene variants in Japanese patients with LVNC. Variants in *MYH7* appear to be a significant cause of LVNC, accounting for almost half of the pathogenic variants identified, whereas the prevalence of *MYBPC3* variants were unexpectedly low. Furthermore, collapsing analysis confirmed that *MYH7* variants increase the risk of developing LVNC, whereas *MYBPC3* variants did not. This genetic spectrum is quite different from previous studies in patients with hypertrophic cardiomyopathy or dilated cardiomyopathy (Table S3). In patients with hypertrophic cardiomyopathy, mutations in *MYBPC3* and *MYH7* are most commonly detected.[17–21] In contrast, in patients with dilated cardiomyopathy, variants in titin are most commonly detected, whereas variants in *MYH7* and *MYBPC3* account for <1%.[22] Although the majority of the LVNC patients presented with the same phenotypic characteristics as patients with dilated cardiomyopathy, heart failure, dilated left ventricle, and decreased LVEF, they have a very different genetic etiology.

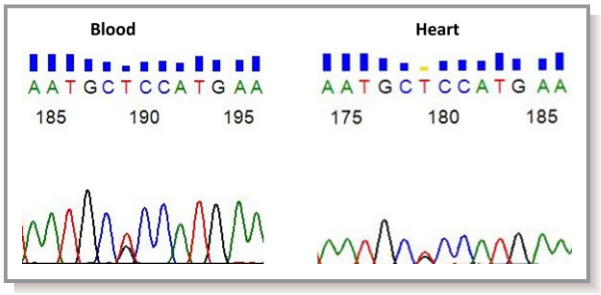

Figure 4. Detection of the *BMPR1A* c.851G>T (p. R284L) variant in DNA isolated from blood and heart samples of a patient with left ventricular noncompaction.

In the patients with *MYH7* variants, we found that there was a broad spectrum in clinical manifestation, ranging from no symptoms to severe heart failure, as reported previously.[9,23] The mechanisms by which *MYH7* variants induce cardiomyopathy are still unclear. Han et al identified abnormal long noncoding RNA transcripts from the *MYH7* locus that may cause cardiomyopathy.[24] Fang et al found that methylation levels in the promoters of *MYH7* may play an important role in regulating embryonic cardiomyocyte gene expression, morphology, and function.[25]

Although previous studies have reported several *MYBPC3* variants in LVNC patients,[9] we identified only 1 pathogenic variant in *MYBPC3,* in a 3-year-old girl. She remained asymptomatic during the 5 years of follow-up. Hypertrophic cardiomyopathy patients with *MYBPC3* mutations also present with reduced or late penetrance, often during the fifth decade of life.[26] Therefore, ongoing follow-up is warranted, even in an asymptomatic patient with LVNC. Among the other sarcomere genes, *ACTC1, TNNT2*,[27] and *TPM1* mutations are less common in LVNC than other cardiomyopathies. *ACTC1* was first reported to be associated with LVNC in 2008,[8] and we reported 2 *TPM1* mutations, as well as 2 *ACTC1* mutations, in LVNC patients in 2011.[28]

TAZ variants may also increase the risk for LVNC, and survival analysis showed worse prognosis in patients with these variants. *TAZ* was identified in 1996 as the causative gene for Barth syndrome,[29] and LVNC is frequently described in patients with Barth syndrome.[30–32] However, half of the patients with *TAZ* variants identified in this study did not show any other manifestations of Barth syndrome. Consequently, male infants with severe heart failure should be considered for genetic analysis, including *TAZ*, even if they do not show any signs of Barth syndrome. In an animal model, tafazzin deficiency leads to ventricular noncompaction and early lethality.[33] Wang et al used induced pluripotent stem cell–derived cardiomyocytes and elucidated that *TAZ* deficiency in Barth syndrome impairs sarcomere assembly and contractile stress generation. *TAZ* deficiency may increase reactive oxygen species production, which may cause features of Barth syndrome.[34]

Among channelopathy-related genes, this is the first report of an *ANK2* variant in LVNC. *ANK2* variants have previously been associated with cardiac arrhythmia syndrome or long QT syndrome and were recently found in hypertrophic cardiomyopathy patients.[35] Although none of our patients who carried variants in arrhythmia-associated genes presented with severe arrhythmias, given the high risk of arrhythmia

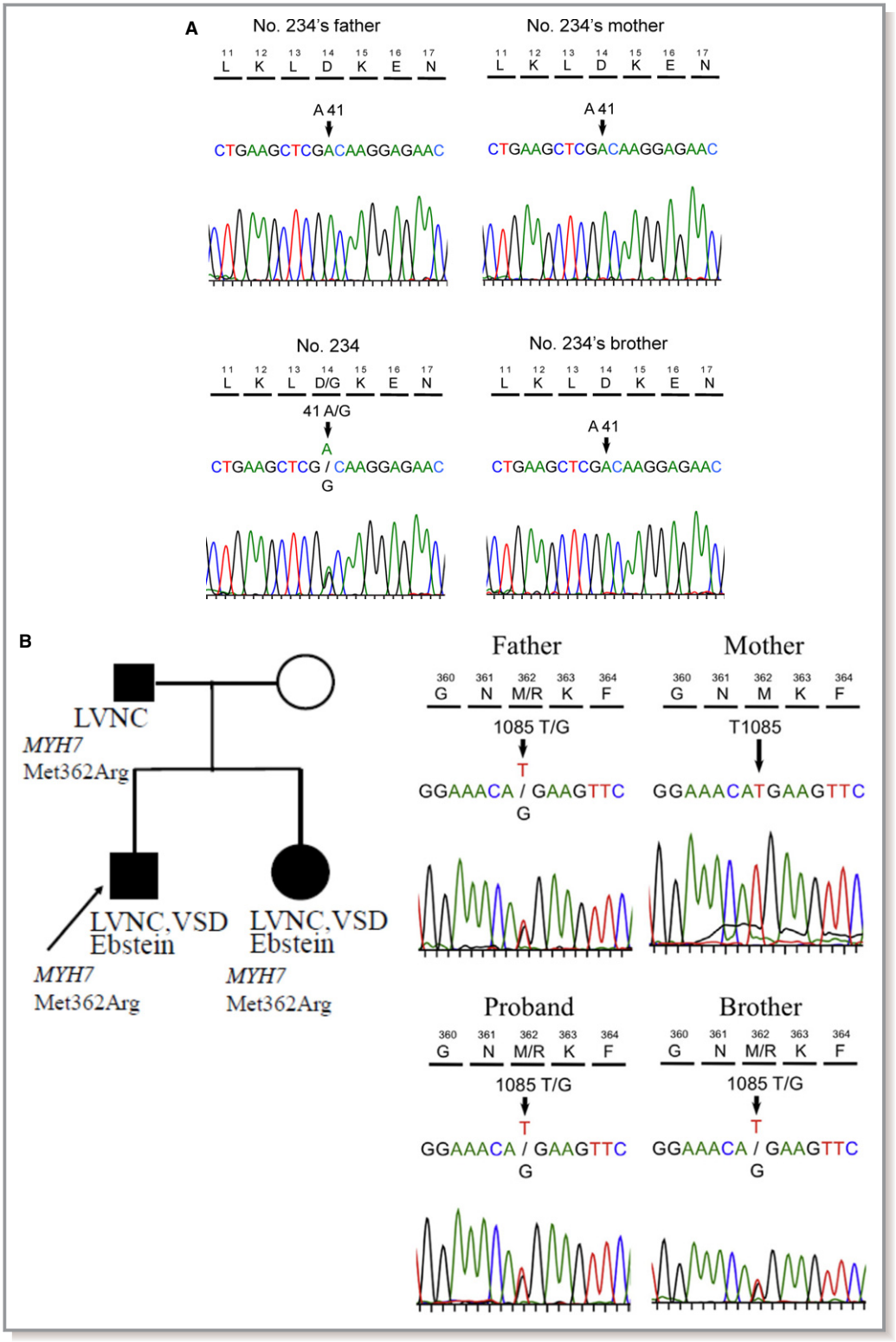

Figure 5. A, De novo variant of *TPM1* c.41A>G (p. D14G) in an LVNC family. B, Familial LVNC and Ebstein anomaly associated with the *MYH7* c.1085T>G p.Met362Arg. LVNC, left ventricular noncompaction.VSD, ventricular septal defect.

associated with these genes, close monitoring and consideration of ICD implantation to prevent sudden cardiac death is recommended.[36]

The variant in *BMPR1A* is also the first reported in a patient with LVNC. BMPs (Bone morphogenetic proteins) are members of the transforming growth factor family that play critical

roles in cardiac development. BMP signaling is required in the myocardium of the atrioventricular canal for proper atrioventricular junction development, and an anomaly in *BMPR1A*-mediated signaling may contribute to the development of cardiac hypertrophy and embryonic heart failure.[37–39] In our study, the patient who carried both *MYH7* and *BMPR1A* variants presented with bradycardia as a fetus and died of heart failure at 1 year of age. Although most patients with a single variant of *MYH7* did not develop severe manifestations, the *BMPR1A* variant may act as genetic modifier and contribute to fetal heart failure. Functional studies of the *BMPR1A* variant are now under way in animal models.

The variant in *TBX5* also represents the first in this gene in a patient with LVNC, as shown in the present study. Both *TBX5* and *TBX20* of the T-box family are important for maintenance of mature cardiomyocyte function.[40,41] Kodo et al showed that proper activation of TGF-β (transforming growth factor β) signaling in the embryonic heart is required to ensure compact layer remodeling. They used patient-specific induced pluripotent stem cell–derived cardiomyocytes generated from an LVNC patient who carried a *TBX20* mutation and found abnormal TGF-β signaling.[41] Functional studies of the *TBX5* mutation are also under way in animal models.

The focused NGS strategy allows for rapid molecular diagnosis at a reasonable cost. In this study, we implemented strict pathogenic variant identification criteria that could prevent misinterpretation of the variants.[42] We found that patients with pathogenic variants showed high morbidity and mortality. Furthermore, patients with double heterozygous variants presented with severe phenotypes during the fetal or neonatal periods and had very poor prognosis, as reported previously.[43] The role of double variants in determining the severity of disease remains unknown and cannot be evaluated using in silico predictive algorithms at the present time. Our study suggests that comprehensive screening of multiple disease-causing genes is necessary to identify high-risk patients with LVNC, for whom earlier treatment strategies toward HT or ICD implantation should be considered.

Limitations

In this study, some parental samples were not available, limiting segregation analysis and the ability to determine whether variants were inherited or arose de novo; none of these patients reported family history, and the parents were healthy and without evidence of cardiomyopathy by ECG and echocardiography. In addition, we chose NGS panels of genes known to be associated with cardiac phenotypes or development; therefore, variants in novel genes would have been missed. Our sequencing approach lacked of ability to assess copy number and structural variants. Whole-exome or -genome sequencing in this cohort might have uncovered additional variants, including copy number variations and structural variants, but at considerably higher cost. Genetic analysis using NGS is considered to have some limitations. Recent studies showed extended genetic noise (false positive), particularly within cardiac disease–associated genes, even if these variants were rare. Guidelines recommend that several in silico analyses be used to evaluate variants without familial and/or experimental evidence of pathogenicity because most algorithms used for missense variant prediction are only 65–80% accurate for known disease variants.[12,44] Further research will be focus on the mechanism presented in animal models and analysis of induced pluripotent stem cells developed from patients with known gene variants to identify the mechanisms that underlie the abnormal development of the failed compacted layer during the embryonic period.

Conclusion

A focused NGS approach revealed a wide and distinct spectrum of gene variants in a large cohort of patients with LVNC. Patients with pathogenic variants showed early age at onset and decreased LVEF. The identification of a pathogenic variant was an independent risk factor for death, HT, or ICD insertion. Survival analysis showed poorer prognosis in the patients with pathogenic variants, especially patients with multiple or *TAZ* variants. Our study suggests that comprehensive screening of multiple disease-causing genes is necessary to identify high-risk patients with LVNC, for whom earlier treatment strategies toward HT or ICD implantation should be considered.

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Disclosures

None.

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SUPPLEMENTAL MATERIAL

Appendix

LVNC Study Collaborators

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Table S1. List of 73 analyzed genes of NGS.

Gene	Chromosome	NCBI Reference Sequence:	Sequence: (Start.End)	
ABCC9	12p12.1	NG_012819.1	NC_000012.11 (21950323..22094797, complement)	http://www.ncbi.nlm.nih.gov/gene/10060
ACTC1	15q14	NG_007553.1	NC_000015.9 (35080297..35087927, complement)	http://www.ncbi.nlm.nih.gov/gene/70
ACTN2	1q42-q43	NG_009081.1	NC_000001.10 (236849754..236927931)	http://www.ncbi.nlm.nih.gov/gene/88
AKAP9	7q21-q22	NG_011623.1	NC_000007.13 (91570181..91739987	http://www.ncbi.nlm.nih.gov/gene/10142
ANK2	4q25-q27	NG_009006.2	NC_000004.11 (113739239..114304896)	http://www.ncbi.nlm.nih.gov/gene/287
BAG3	10q25.2-q26.2	NG_016125.1	NC_000010.10 (121410859..121437331)	http://www.ncbi.nlm.nih.gov/gene/9531
BMPR1A	10q22.3	NG_009362.1	NC_000010.10 (88516396..88684945)	http://www.ncbi.nlm.nih.gov/gene/657
CACNA1C	12p13.3	NG_008801.2	NC_000012.11 (2079952..2807115)	http://www.ncbi.nlm.nih.gov/gene/775
CACNB2	10p12	NG_016195.1	NC_000010.10 (18429373..18830688)	http://www.ncbi.nlm.nih.gov/gene/783

CALR3	19p13.11	NG_031959.2	NC_000019.9 (16589767..16607015, complement)	http://www.ncbi.nlm.nih.gov/gene/125972
CAPN3	15q15.1	NG_008660.1	NC_000015.9 (42646545..42704515)	http://www.ncbi.nlm.nih.gov/gene/825
CAV3	3p25	NG_008797.2	NC_000003.11 (8775486..8788451)	http://www.ncbi.nlm.nih.gov/gene/859
COL4A1	13q34	NG_011544.1	NC_000013.10 (110801310..110959496, complement)	http://www.ncbi.nlm.nih.gov/gene/1282
DES	2q35	NG_008043.1	NC_000002.11 (220283099..220291461)	http://www.ncbi.nlm.nih.gov/gene/1674
DMD	Xp21.2	NG_012232.1	NC_000023.10 (31137345..33357726, complement)	http://www.ncbi.nlm.nih.gov/gene/1756
DSC2	18q12.1	NG_008208.1	NC_000018.9 (28645938..28682388, complement)	http://www.ncbi.nlm.nih.gov/gene/1824
DSG2	18q12.1	NG_007072.3	NC_000018.9 (29078027..29128814)	http://www.ncbi.nlm.nih.gov/gene/1829
DSP	6p24	NG_008803.1	NC_000006.11 (7541808..7586946)	http://www.ncbi.nlm.nih.gov/gene/1832
ELN	7q11.23	NG_009261.1	NC_000007.13 (73442119..73484237)	http://www.ncbi.nlm.nih.gov/gene/2006
EMD	Xq28	NG_008677.1	NC_000023.10 (153607597..153609883)	http://www.ncbi.nlm.nih.gov/gene/2010
GAA	17q25.2-q25.3	NG_009822.1	NC_000017.10 (78075339..78093680)	http://www.ncbi.nlm.nih.gov/gene/2548

GATA4	8p23.1-p22	NG_008177.1	NC_000008.10 (11534433..11617510)	http://www.ncbi.nlm.nih.gov/gene/2626
GLA	Xq22	NG_007119.1	NC_000023.10 (100652779..100663001, complement)	http://www.ncbi.nlm.nih.gov/gene/2717
GPD1L	3p22.3	NG_023375.1	NC_000003.11 (32148003..32210207)	http://www.ncbi.nlm.nih.gov/gene/23171
HCN4	15q24.1	NG_009063.1	NC_000015.9 (73612200..73661605, complement)	http://www.ncbi.nlm.nih.gov/gene/10021
JUP	17q21	NG_009090.2	NC_000017.10 (39910859..39942964, complement)	http://www.ncbi.nlm.nih.gov/gene/3728
KCNE1	21q22.12	NG_009091.1	NC_000021.8 (35790910..35884573, complement)	http://www.ncbi.nlm.nih.gov/gene/3753
KCNE2	21q22.12	NG_008804.1	NC_000021.8 (35736323..35743440)	http://www.ncbi.nlm.nih.gov/gene/9992
KCNE3	11q13.4	NG_011833.1	NC_000011.9 (74165886..74178600, complement)	http://www.ncbi.nlm.nih.gov/gene/10008
KCNH2	7q36.1	NG_008916.1	NC_000007.13 (150642044..150675402, complement)	http://www.ncbi.nlm.nih.gov/gene/3757
KCNJ2	17q24.3	NG_008798.1	NC_000017.10 (68164757..68176189)	http://www.ncbi.nlm.nih.gov/gene/3759
KCNQ1	11p15.5	NG_008935.1	NC_000011.9 (2466221..2870340)	http://www.ncbi.nlm.nih.gov/gene/3784

KRAS	12p12.1	NG_007524.1	NC_000012.11 (25358180..25403870, complement)	http://www.ncbi.nlm.nih.gov/gene/3845
LAMP2	Xq24	NG_007995.1	NC_000023.10 (119560003..119603204, complement)	http://www.ncbi.nlm.nih.gov/gene/3920
LDB3	10q22.3-q23.2	NG_008876.1	NC_000010.10 (88426542..88495829)	http://www.ncbi.nlm.nih.gov/gene/11155
LMNA	1q22	NG_008692.2	NC_000001.10 (156052369..156109880)	http://www.ncbi.nlm.nih.gov/gene/4000
MYBPC3	11p11.2	NG_007667.1	NC_000011.9 (47352957..47374253, complement)	http://www.ncbi.nlm.nih.gov/gene/4607
MYH11	16p13.11	NG_009299.1	NC_000016.9 (15796992..15950887, complement)	http://www.ncbi.nlm.nih.gov/gene/4629
MYH6	14q12	NG_023444.1	NC_000014.8 (23849942..23878836, complement)	http://www.ncbi.nlm.nih.gov/gene/4624
MYH7	14q12	NG_007884.1	NC_000014.8 (23881947..23904870, complement)	http://www.ncbi.nlm.nih.gov/gene/4625
MYL2	12q24.11	NG_007554.1	NC_000012.11 (111348623..111358404, complement)	http://www.ncbi.nlm.nih.gov/gene/4633
MYL3	3p21.3-p21.2	NG_007555.2	NC_000003.11 (46899357..46904973, complement)	http://www.ncbi.nlm.nih.gov/gene/4634

MYLK	3q21	NG_029111.1	NC_000003.11 (123331143..123603149, complement)	http://www.ncbi.nlm.nih.gov/gene/4638
MYOZ2	4q26-q27	NG_029747.1	NC_000004.11 (120056939..120108944)	http://www.ncbi.nlm.nih.gov/gene/51778
NKX2-5	5q34	NG_013340.1	NC_000005.9 (172659107..172662315, complement)	http://www.ncbi.nlm.nih.gov/gene/1482
NRAS	1p13.2	NG_007572.1	NC_000001.10 (115247085..115259515, complement)	http://www.ncbi.nlm.nih.gov/gene/4893
PKP2	12p11	NG_009000.1	NC_000012.11 (32943680..33049780, complement)	http://www.ncbi.nlm.nih.gov/gene/5318
PLN	6q22.1	NG_009082.1	NC_000006.11 (118869442..118881587)	http://www.ncbi.nlm.nih.gov/gene/5350
PRKAG2	7q36.1	NG_007486.1	NC_000007.13 (151253200..151574316, complement)	http://www.ncbi.nlm.nih.gov/gene/51422
PTPN11	12q24	NG_007459.1	NC_000012.11 (112856536..112947717)	http://www.ncbi.nlm.nih.gov/gene/5781
RAF1	3p25	NG_007467.1	NC_000003.11 (12625100..12705700, complement)	http://www.ncbi.nlm.nih.gov/gene/5894
RPS7	2p25	NG_011744.1	NC_000002.11 (3622853..3628509)	http://www.ncbi.nlm.nih.gov/gene/6201

RYR2	1q43	NG_008799.2	NC_000001.10 (237205510..237997288)	http://www.ncbi.nlm.nih.gov/gene/6262
SCN1B	9q13.1	NG_013359.1	NC_000019.9 (35521555..35531353)	http://www.ncbi.nlm.nih.gov/gene/6324
SCN3B	11q23.3	NG_016283.1	NC_000011.9 (123499895..123525315, complement)	http://www.ncbi.nlm.nih.gov/gene/55800
SCN4B	11q23.3	NG_011710.1	NC_000011.9 (118004092..118023630, complement)	http://www.ncbi.nlm.nih.gov/gene/6330
SCN5A	3p21	NG_008934.1	NC_000003.11 (38589553..38691164, complement)	http://www.ncbi.nlm.nih.gov/gene/6331
SGCD	5q33-q34	NG_008693.2	NC_000005.9 (155462147..156194799)	http://www.ncbi.nlm.nih.gov/gene/6444
SLC25A4	4q35	NG_013001.1	NC_000004.11 (186064417..186071538)	http://www.ncbi.nlm.nih.gov/gene/291
SMAD3	15q22.33	NG_011990.1	NC_000015.9 (67358036..67487533)	http://www.ncbi.nlm.nih.gov/gene/4088
SNTA1	20q11.2	NG_011622.1	NC_000020.10 (31995763..32031698, complement)	http://www.ncbi.nlm.nih.gov/gene/6640
SOS1	2p21	NG_007530.1	NC_000002.11 (39208690..39347686, complement)	http://www.ncbi.nlm.nih.gov/gene/6654
STARD3	17q11-q12		NC_000017.10 (37793333..37820454)	http://www.ncbi.nlm.nih.gov/gene/10948
TAZ	Xq28	NG_009634.1	NC_000023.10 (153639877..153650065)	http://www.ncbi.nlm.nih.gov/gene/6901

TBX5	12q24.1	NG_007373.1	NC_000012.11 (114791735..114846247, complement)	http://www.ncbi.nlm.nih.gov/gene/6910
TGFBR1	9q22	NG_007461.1	NC_000009.11 (101867412..101916474)	http://www.ncbi.nlm.nih.gov/gene/7046
TGFBR2	3p22	NG_007490.1	NC_000003.11 (30647994..30735634)	http://www.ncbi.nlm.nih.gov/gene/7048
TMEM43	3p25.1	NG_008975.1	NC_000003.11 (14166440..14185180)	http://www.ncbi.nlm.nih.gov/gene/79188
TNNC1	3p21.1	NG_008963.1	NC_000003.11 (52485107..52488057, complement)	http://www.ncbi.nlm.nih.gov/gene/7134
TNNI3	19q13.4	NG_007866.2	NC_000019.9 (55663135..55669100, complement)	http://www.ncbi.nlm.nih.gov/gene/7137
TNNT2	1q32	NG_007556.1	NC_000001.10 (201328136..201346836, complement)	http://www.ncbi.nlm.nih.gov/gene/7139
TPM1	15q22.1	NG_007557.1	NC_000015.9 (63334838..63364114)	http://www.ncbi.nlm.nih.gov/gene/7168
VCL	10q22.2	NG_008868.1	NC_000010.10 (75757836..75879918)	http://www.ncbi.nlm.nih.gov/gene/7414

Table S2. Silico predictive algorithms used in the study.

Category	Basis	Name	Website	Prediction Threshold
Missense prediction	Evolutionary conservation	FATHMM	http://fathmm.biocompute.org.uk	<-1.5 Damaging >-1.5 Tolerated
		SIFT	http://sift.jcvi.org	<0.05 Deleterious >0.05 Tolerated
Missense prediction	Protein structure/function and evolutionary conservation	Align GVGD	http://agvgd.iarc.fr/agvgd_input.php	≧C15 Probably Damaging
		Mutation Taster	http://www.mutationtaster.org	Disease causing
		Polyphen-2	http://genetics.bwh.harvard.edu/pph2	≧0.432 Possibly Damaging ≧0.85 Probably Damaging

Missense and insertion/ deletions prediction	Alignment and measurement of similarity between variant sequence and protein sequence homolog	PROVEAN	http://provean.jcvi.org/index.php	<-2.5 Deleterious >-2.5 Neutral
Missense and insertion/ deletions prediction	Contrasts annotations of fixed/nearly fixed derived alleles in humans with simulated variants	CADD	http://cadd.gs.washington.edu	≧20 1% most deleterious ≧30 0.1%most deleterious

Reference

1.Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the association for molecular autopsy. Genet Med 2015; 17:405–423.

Table S3. Frequency of MYH7 and MYBPC3 in LVNC, HCM and DCM patients.

Gene	% Frequency of mutations in LVNC patients in this study (n=102)	% Frequency of mutations in HCM in Japanese cohort* (n=127)	% Frequency of mutations in HCM in French cohort† (n=172)	% Frequency of mutations in HCM in US cohort study‡ (n=389)	% Frequency of mutations in DCM in Finnish cohort study§ (n=145)
MYH7	19.6	24.4	26.2	15.2	0.7
MYBPC3	0.98	15	26.2	18	0

* *Heart Vessels.* (2016). doi:10.1007/s00380-016-0920-0. † *Circulation* 2003; 107: 2227–2232. ‡*J Am Coll Cardiol* 2004; 44: 1903–1910.

§ *Eur Heart J*. 2015;36(34):2327-2337.

Table S4. Novel mutations, absent in Exome Aggregation Consortium and Human Genetic Variation Database (HGVD).

		Prediction						
Gene	**variant**	**FATHMM**	**SIFT**	**Polyphen2**	**Align GVGD**	**Mutation Taster**	**Provean**	**CADD**
MYH7	R941C	Damaging Score: -2.13	Deleterious Score:0	Probably Damaging Score:1	C65	Disease causing	Deleterious Score: -6.13	34
	Q315R	Damaging Score: -2.33	Deleterious Score:0.011	Possibly Damaging Score:0.51	C0	Disease causing	Deleterious Score: -3.16	23.8
	F230S	Damaging Score: -4.96	Deleterious Score:0	Probably Damaging Score:0.984	C0	Disease causing	Deleterious Score: -6.07	27.7
	K542N	Damaging Score: -2.47	Deleterious Score:0	Probably Damaging Score:1	C65	Disease causing	Deleterious Score: -4.4	31

A223V	Damaging	Deleterious	Probably Damaging	C0	Disease causing	Deleterious	25.1
	Score: -3.17	Score:0.08	Score:0.854			Score: -2.75	
M362R	Damaging	Deleterious	Benign	C0	Disease causing	Deleterious	26.9
	Score: -3.64	Score:0	Score:0.001			Score: -5.15	
K542T	Damaging	Deleterious	Possibly Damaging	C65	Disease causing	Deleterious	27.4
	Score: -2.48	Score:0	Score:0.517			Score: -4.4	
E667V	Damaging	Tolerated	Probably Damaging	C65	Disease causing	Deleterious	26.1
	Score: --2.46	Score:0.113	Score:0.994			Score: -5.14	
E448K	Damaging	Deleterious	Possibly Damaging	C0	Disease causing	Deleterious	32
	Score: -2.22	Score:0.002	Score:0.798			Score: -2.61	
L693R	Damaging	Deleterious	Probably Damaging	C65	Disease causing	Deleterious	28.3
	Score: -4.85	Score:0	Score:0.997			Score: -5.29	
R712H	Damaging	Deleterious	Probably Damaging	C25	Disease causing	Deleterious	35

		Score: -4.54	Score:0	Score:0.988			Score: -4.35	
	c.896-1 G>A	NA	NA	NA	NA	NA	NA	24.9
TAZ	Q159P	Damaging	Deleterious	Probably Damaging	C0	Disease causing	Deleterious	23.4
		Score: -4.39	Score:0.001	Score:0.993			Score: -5.73	
	M185V	Damaging	Deleterious	Probably Damaging	C0	Disease causing	NA	26.6
		Score: -3.16	Score:0.03	Score:0.932				
	L169F	Damaging	Deleterious	Probably Damaging	C0	Disease causing	Deleterious	31
		Score: -4.78	Score:0.01	Score:0.886			Score: -3.33	
	H176Y	Damaging	Deleterious	Probably Damaging	C0	Disease causing	NA	16.57
		Score: -3.15	Score:0	Score:0.999				
ACTC1	T231R	Damaging	NA	Probably Damaging	C65	Disease causing	Deleterious	24.9
		Score: -4.39		Score:0.908			Score: -2.65	
	Y93H	Damaging	NA	Possibly Damaging	C65	Disease causing	Deleterious	24

		Score: - 3.43		Score:0.531			Score: -3.59	
TPM1	R238Q	Damaging	Deleterious	Probably Damaging	C35	Disease causing	Deleterious	35
		Score: -6.36	Score:0.001	Score:0.999			Score: -3.22	
	D14G	Damaging	Deleterious	Probably Damaging	C0	Disease causing	Deleterious	29.9
		Score: -2.38	Score:0.001	Score:1			Score: -3.21	
MYL2	E88K	Tolerated	Deleterious	Probably Damaging	C15	Disease causing	Deleterious	34
		Score: -1.15	Score:0.017	Score:0.995			Score: -3.62	
TNNC1	E94A	Damaging	Deleterious	Benign	C65	Disease causing	Deleterious	24.2
		Score: -3.74	Score:0	Score:0.012			Score: -5.36	
MYBPC3	G758D	Tolerated	Deleterious	Probably Damaging	C65	Disease causing	Deleterious	32
		Score: -1.64	Score:0.001	Score:0.926			Score: -5.96	
LMNA	A244V	Damaging	Deleterious	Probably Damaging	C65	Disease causing	Deleterious	34
		Score: -2.5	Score:0.001	Score:1			Score: -3.76	

SGCD	N99H*	Damaging Score: -3.45	Deleterious Score:0.05	Possibly Damaging Score:0.744	C0	Disease causing	Neutral Score: -0.69	23.4
BMPR1A	R284L	Damaging Score: -3.32	Deleterious Score:0	Probably Damaging Score:0.988	C65	Disease causing	Deleterious Score: -6.74	35
HCN4	G480S	Damaging Score: -7.52	Deleterious Score:0.024	Probably Damaging Score:1	C55	Disease causing	Deleterious Score: -5.74	25.9
TBX5†	p. Arg279Ter	NA	NA	NA	C25	Disease causing	NA	40

The classification of novel variants is all likely pathogenic except TBX5 p. Arg279Ter. ***** Two patients have this variant. † Nonsense mutation and classification is pathogenic.

Table S5. Gene collapsing test of rare variants.

Rank	Gene	Qualifying Cases	Frequency Qualifying Cases (N=102)	Qualifying Controls Cases	Frequency Qualifying Controls (N=4327)	Fisher's Exact Test p-value
1	MYH7	19	0.1862	41	0.0095	1.29 E-17
2	TAZ	6	0.0588	2	0.0005	3.48 E-9
3	MYL2	2	0.0196	5	0.0012	0.01
4	ACTC1	2	0.0196	2	0.0005	0.003
5	TPM1	2	0.0196	2	0.0005	0.003
6	SGCD	2	0.0196	5	0.0012	0.01
7	ANK2	1	0.0098	4	0.0009	0.251
8	TNNC1	1	0.0098	5	0.0012	0.131
9	BMPR1A	1	0.0098	5	0.0012	0.131
10	KCNE3	1	0.0098	6	0.0014	0.151
11	TBX5	1	0.0098	7	0.0016	0.170
12	HCN4	1	0.0098	8	0.0018	0.193
13	LMNA	1	0.0098	9	0.0021	0.208
14	KCNH2	1	0.0098	20	0.0046	0.388
15	MYBPC3	1	0.0098	35	0.0081	0.569
16	JUP	1	0.0098	37	0.0086	0.589

Table S6. Specific variants found in subjects with systolic dysfunction versus those without dysfunction

	with systolic dysfunction			without systolic dysfunction	
ID	Gene	variant	ID	Gene	variant
132	MYL2	E88K	250	MYH7	E677V
133	ACTC1	Y93H	298	MYH7	R904C
143	MYH7	E1801K	401	HCN4	G480S
153	MYH7	E448K			
159	TAZ	c.109+1G>C			
233	KCNH2	A561T			
247	MYH7	R712H			
260	SGCD	N99H			
309	MYH7	M362R			
312	ACTC1	T231R			
313	TAZ	M185V			
315	MYBPC3	G758D			
321	TNN1C	E94A			
327	TAZ	L169F			
333	MYH7	A223V			
341	ANK2	R321W			
350	TPM1	R238Q			
361	MYH7	c.896-1 G>A			
362	MYH7	F230S			
365	MYL2	P144fs			
377	MYH7	L693R			
378	MYH7	L620P			
386	TBX5	p. Arg279Ter			

390	MYH7	E1914K
391	MYH7	E1801K
392	MYH7	Q315R
415	TAZ	Q159P
427	MYH7	R941C
403	TAZ	G197R
404	MYH7	R23W
405	LMNA	A244V
342	MYH7	P838L
	BMPR1A	R284L
280	MYH7	K542N
	JUP	E146K
274	KCNE3	R99H
	TAZ	H176Y
339	ANK2	W3620R
	MYH7	K542N
234	SGCD	N99H
	TPM1	D14G

Table S7. Details for each subject.

ID	Gene	variant	Sex (1M 2F)	age on set	Heart failure	family history (0 no)	LVEF%	LVDD-Z SCORE	Arrhythmia (0 normal)	Prognosis (0: alive)
132	MYL2	E88K	1	0.083	1	father DCM	39	1.2	0	0
133	ACTC1	Y93H	2	0	1	Mother LVNC	36	1.88	0	0
143	MYH7	E1801K	1	0	1	0	42	1	0	0
153	MYH7	E448K	2	0.083	0	Sister LVNC	46	0.795	0	0
159	TAZ	c.109+1G>C	1	0.167	1	Mother LVNC	20	2.888	0	death
233	KCNH2	A561T	1	0	1	0	31	1.1116	non-specific change	death
247	MYH7	R712H	2	0	1	0	36	-0.244	0	Heart transplantation

250	MYH7	E677V	1	0.04	1	0	60	1.6326	0	0
260	SGCD	N99H	1	0.083	0	0	45	1.5	0	0
298	MYH7	R904C	2	6	0	0	65	2.1	0	0
309	MYH7	M362R	2	0.01	0	Father and brother LVNC	39	1.08	0	0
312	ACTC1	T231R	1	4	1	0	49.4	1.8727	Supraventricular tachycardia	ICD
313	TAZ	M185V	1	0.083	1	Mother LVNC	40	3.3333	0	Heart transplantation
315	MYBPC3	G758D	2	3	0	0	38.5	1.25	0	0
321	TNN1C	E94A	2	0.333	0	0	32.5	2.22	0	Heart transplantation
327	TAZ	L169F	1	0	1	brother LVNC	22	2.566	0	0

333	MYH7	A223V	1	0	1	0	10	-0.4	0	0
339	MYH7	K542N	2	0	1	0	48	1	non-specific change	0
341	ANK2	R321W	2	0.083	1	0	46.8	1.9166	non-specific change	0
350	TPM1	R238Q	2	0	1	0	38.4	1.33	0	0
361	MYH7	c.896-1 G>A	2	0	1	0	38	0.977	0	0
362	MYH7	F230S	2	0.0833	0	0	40	-0.823	0	0
365	MYL2	P144fs	2	0.0416	1	0	34	1.7391	0	0
377	MYH7	L693R	2	0.0833	1	0	30	1.3333	0	0
378	MYH7	L620P	2	0	1	Sister LVNC	20	0.1	AF	0
386	TBX5	p. Arg279Ter	1	0	1	0	31	1.88	non-specific change	0
390	MYH7	E1914K	1	0	0	0	43.5	3.3833	0	0
391	MYH7	E1801K	2	0.8333	1	0	38	2.6785	0	0

392	MYH7	Q315R	1	0.0833	1	0	29	2.1428	T wave	0
415	TAZ	Q159P	1	0.8333	1	Sister LVNC	14	2.4	0	0
427	MYH7	R941C	2	1.5	0	father DCM	44.1	1.3709	0	0
401	HCN4	G480S	2	0.0166	1	Mother LVNC	59.4	-0.833	0	0
403	TAZ	G197R	1	0.25	1	0	6	4.4523	0	0
404	MYH7	R23W	2	0.08333	0	0	48.9	0.0408	0	0
405	LMNA	A244V	2	0.08333	1	Sister LVNC	34	1.89	0	0
342 342	MYH7 BMPR1A	P838L R284L	2	0	1	0	38	1.72	ventricular fibrillation	death
280 280	MYH7 JUP	K542N E146K	1	0	1	0	40	2.16	0	death
274	KCNE3	R99H	1	0.005	1	0	45	2.03	left bundle branch	death

274	TAZ	H176Y							block	
234 234	SGCD TPM1	N99H D14G	1	0	1	0	27	3.3095	0	death