ORIGINAL RESEARCH

Genotype-Positive Status Is Associated With Poor Prognoses in Patients With Left Ventricular Noncompaction Cardiomyopathy

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***Background*—**Left ventricular noncompaction cardiomyopathy (LVNC) is a genetically and phenotypically heterogeneous disease. This study aims to investigate the genetic basis and genotype-phenotype correlations in a cohort of Chinese patients with LVNC.

***Methods and Results*—**A total of 72 cardiomyopathy-associated genes were comprehensively screened in 83 adults and 17 children with LVNC by targeted sequencing. Pathogenicity of the detected variants was determined according to their prevalence and American College of Medical Genetics and Genomics recommendations. Baseline and follow-up clinical data were collected. The primary end point was a composite of death and heart transplantation. Overall, 42 pathogenic variants were identified in 38 patients (38%), with *TTN*, *MYH7*, *MYBPC3*, and *DSP* being the most commonly involved genes. At baseline, genotype-positive adults had higher rates of atrial fibrillation and family history, and lower left ventricular ejection fraction, compared with genotype-negative adults. During a median follow-up of 4.2 years, more primary end points occurred in genotype-positive adults than in genotype-negative adults (50.0% versus 23.5%; P=0.013). Multivariable analysis demonstrated that genotype-positive status was associated with higher risks of death and heart transplantation, independent of age, sex, and cardiac function at baseline in patients with LVNC (adjusted hazards ratio, 2.49; 95% confidence interval, 1.15–5.37; P=0.020).

***Conclusions*—**Our study revealed a distinct genetic spectrum in Chinese patients with LVNC, with variants in *TTN*, *MYH7*, *MYBPC3*, and *DSP* being the most common. The presence of pathogenic variants is an independent risk factor for adverse outcomes and may aid in risk stratification in adult patients. Larger studies are needed to confirm these findings. **(*J Am Heart Assoc*. 2018;7: e009910. DOI: 10.1161/JAHA.118.009910.)**

Key Words: genetics • left ventricular noncompaction • prognosis

Left ventricular noncompaction cardiomyopathy (LVNC) is a genetically and clinically heterogeneous myocardial disorder that is present in 3% to 4% of patients with heart failure (HF).[1,2] It is characterized by a prominent trabecular meshwork and deep intertrabecular recesses communicating with the left ventricular cavity, morphologically reminiscent of early cardiac development.[3,4] The genesis of LVNC is generally thought to be caused by the arrest of myocardial compaction during the normal development of the heart.[5,6] Patients with LVNC show a wide spectrum of clinical presentations, ranging from no symptoms to thromboembolic events, end-stage HF, or sudden cardiac death (SCD).[7–10]

LVNC was classified as a genetic cardiomyopathy by the American Heart Association,[11] and genetics plays an important role in it. Genetic defects are found in 35% to 40% of patients with isolated LVNC by molecular testing, with *MYH7* as the most commonly involved gene.[12,13] Genes associated with LVNC usually include those encoding sarcomeric, cytoskeletal, or ion channel proteins, and those involved in cellular energy metabolism.[4]

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Accompanying Data S1 and Tables S1 through S8 are available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.118.009910

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Clinical Perspective

What Is New?

- In this large cohort study, we described a distinct genetic spectrum in Chinese patients with left ventricular noncompaction cardiomyopathy.
- More than one third of the patients carried at least one pathogenic variant, with *TTN*, *MYH7*, *MYBPC3*, and *DSP* being the most commonly involved genes.
- Genotype-positive status was independently associated with increased risk of death and heart transplantation in adult patients.

What Are the Clinical Implications?

- In the context of left ventricular noncompaction cardiomyopathy, genetic testing has a considerable yield in Chinese patients, as previously described in patients of European ancestry.
- Genetic testing is important for better risk stratification of the individual patient and should be recommended in clinical practice.

Although LVNC has been studied for nearly a century since it was first described in 1926,[14] there is still a lot to be clarified about its genetic basis and genotype-phenotype correlations. Thus, the current study aims to investigate the molecular defects, clinical manifestations, and long-term outcomes, as well as the relationships among them, in a cohort of Chinese patients with LVNC.

Methods

Because of privacy, the data, analytic methods, and study materials will not be made available to other researchers for purposes of reproducing the results or replicating the procedure.

Study Subjects

A total of 100 unrelated patients with LVNC enrolled at Fuwai Hospital between April 2004 and May 2016 were included in the study. LVNC was diagnosed on the basis of established criteria from echocardiography and/or cardiac magnetic resonance.[15,16] The core item of these criteria is the ratio of the thickness of noncompacted/compact epicardial layer, which is >2.0 measured on echocardiography in systole or >2.3 on cardiac magnetic resonance in diastole. According to the age of onset, patients were divided into children (<18 years) and adults (≥18 years). Demographic and clinical data of all participants were collected.

The study complies with the principles of the Declaration of Helsinki and was approved by the Ethics Committee of Fuwai Hospital. Written informed consent was obtained from each participant.

Targeted Sequencing

Genomic DNA was isolated from peripheral venous blood of each participant. The coding exons and their splicing regions (adjacent 10-bp intronic sequences) of 72 cardiomyopathy-related genes were enriched, as determined using a custom-designed library (Agilent Technologies, Santa Clara, CA), and were subsequently sequenced on an Illumina next-generation sequencing platform (Illumina Inc, San Diego, CA). Sequencing reads of each individual were mapped to the human reference genome with BWA (0.7.12). After removal of polymerase chain reaction duplications with PICARD, variants were called with GATK, version 3.5, and annotated by using ANNOVAR. The mean depths of the samples were >400×, with coverage of >99.7%. Details about the genes being tested are described in Data S1.

Variants were described in accordance with the guidelines for mutation nomenclature of the Human Genome Variation Society (http://www.hgvs.org/). Variants were considered as polymorphisms and excluded if their minor allele frequency was ≥0.05% among East Asians in the Genome Aggregation Database.[17] The pathogenicity of detected variants was determined in accordance with the recommendations of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology, and was classified as "pathogenic," "likely pathogenic," "uncertain significance," "likely benign," or "benign."[18] Criteria for classification are listed in Table S1 and S2. Variants classified as pathogenic or likely pathogenic were considered to be pathogenic in the current analysis. Patients with or without pathogenic variants were defined as genotype positive (G+) or genotype negative (G−), respectively. Sanger sequencing was used to validate the presence of pathogenic variants. The primers for the sequencing are listed in Table S3.

Follow-Up and End Points

Follow-up data were obtained by telephone interview or clinic visit. The last follow-up was performed in April 2018. The primary end point was a composite of death and heart transplantation (HT). The secondary end points included all-cause death, HT, and cardiovascular death, which included SCD, HF-related death, and death from other cardiovascular causes. SCD was defined as witnessed sudden death with or without documented ventricular fibrillation, death within 1 hour of new symptoms, or nocturnal deaths with no antecedent history of worsening symptoms. HF-related death

was defined as death preceded by symptoms of HF lasting >1 hour.

Statistical Analysis

Continuous variables are expressed as median (interquartile range) and were compared by independent-sample *t* test or Mann-Whitney *U* test for abnormally distributed variables. Categorical variables are presented as proportions (%) and were compared using Pearson's χ^2 test or Fisher's exact test. Univariable or multivariable Cox proportional hazards models were performed to calculate the hazard ratio and 95% confidence interval to estimate the effect of pathogenic variants on phenotypes. Survival curves were constructed in accordance with the Kaplan-Meier method, and were compared using the log-rank test. Factors included in the multivariate models for the outcomes were age, sex, and New York Heart Association functional class III/IV at baseline. Differences were considered significant if the 2-sided *P* value was <0.05. All analyses were performed with SPSS, version 22.0 software (IBM, Armonk, NY).

Results

Study Population and Genetic Profile

The participants in this study consisted of 17 children (17%) and 83 adults (83%). A total of 72 (72%) of them were male, with a median (interquartile range) age of 42 (24–53) years. A total of 42 pathogenic variants were found in 38 (38.0%) of patients, of which 29 (69.0%) of variants were located in sarcomere genes (Table S4). Among these sarcomere genes, *TTN* was the most commonly involved gene (51.7%), followed by *MYH7* (17.2%), *MYBPC3* (13.8%), and *ACTC1* (6.9%) (Figure 1A). Eight nonsarcomere genes were detected with pathogenic variants, among which *DSP* (23.1%) was the most commonly mutated gene, followed by *DMD* (15.4%), *LAMP2* (15.4%), and *SCN5A* (15.4%). Notably, 25 (59.5%) of detected variants were novel, which were located in *TTN*, *MYBPC3*, *DSP*, *NNT*, *DMD*, and *LAMP2*.

There was no significant difference in the proportions of G+ status between children (35.3%, 6/17) and adults (38.6%, 32/83; *P*=0.801). The proportions of variants in sarcomere genes were not significantly different between children and adults (42.9% and 72.2%, respectively; *P*=0.190), with *TTN* being the predominant gene in both groups (Figure 1B and 1C). Nonsarcomere genes accounted for 57.1% and 27.8% of the variants detected in children and adults, respectively.

Genotype-Phenotype Correlation at Baseline

Demographic and clinical characteristics of the participants are shown in Table 1. Similar characteristics were observed between G+ and G− children. For adults, atrial fibrillation and a family history of cardiomyopathy were more common; left ventricular ejection fraction was lower in G+ patients, whereas G− patients were more likely to have hypertension. Notably, 6 carriers of pathogenic variants in *DSP* were all found to have ventricular arrythmias (Table S5).

Genotype-Phenotype Correlation for Clinical Outcomes

During a median (interquartile range) follow-up of 4.5 (2.9–6.2) years, 32 patients (32.0%) reached the primary end point,

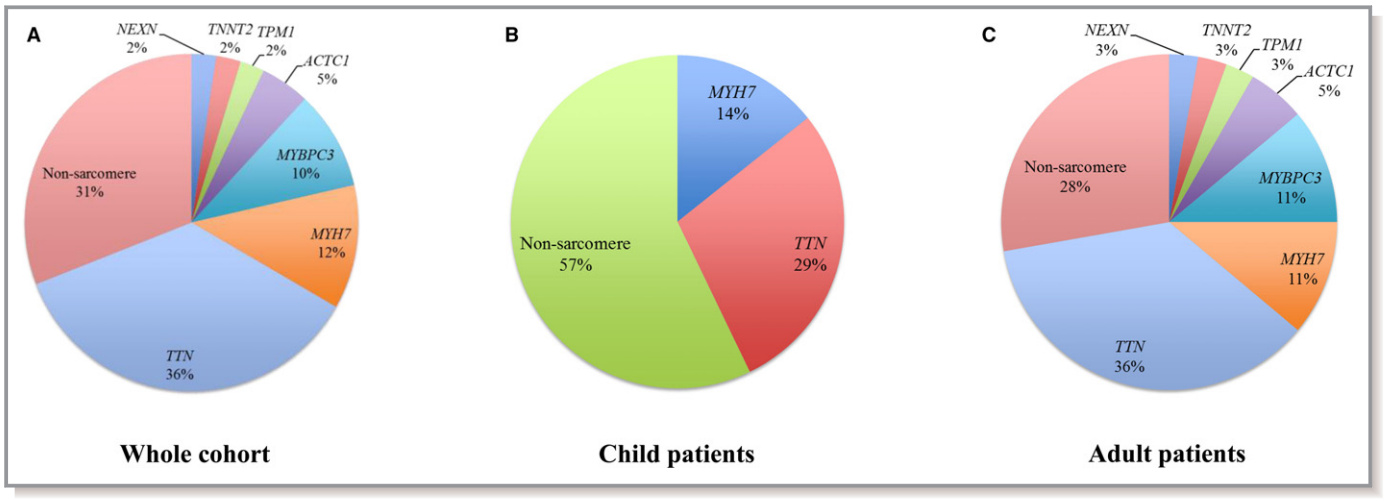

Figure 1. Spectrum of pathogenic variants in Chinese patients with left ventricular noncompaction cardiomyopathy. Panels show the variant spectrum in whole cohort (A), child patients (B) and adult patients (C).

Table 1. Baseline Characteristics of G+ and G− Among Children and Adults

Characteristics	Children (n=17)			Adults (n=83)		
	G+ (n=6)	G− (n=11)	*P* Value	G+ (n=32)	G− (n=51)	*P* Value
Age at enrollment, y	16.0 (13.0–18.0)	14.0 (8.0–15.0)	0.149	44.0 (35.5–49.0)	46.0 (30.0–57.0)	0.506
Age of onset, y	15.5 (10.5–17.0)	13.0 (8.0–15.0)	0.180	39.5 (30.3–45.5)	42.0 (28.0–52.0)	0.660
Male sex, n (%)	6 (100.0)	8 (72.7)	0.515	22 (68.8)	36 (70.6)	0.859
Family history of cardiomyopathy, n (%)	1 (16.7)	1 (9.1)	1.000	9 (28.1)	2 (3.9)	0.002
NYHA class III/IV, n (%)	3 (50.0)	1 (9.1)	0.099	17 (53.1)	22 (43.1)	0.375
Comorbidities, n (%)						
Coronary artery disease	0 (0.0)	0 (0.0)	···	1 (3.1)	8 (15.7)	0.143
Hypertension	0 (0.0)	1 (9.1)	1.000	0 (0.0)	13 (25.5)	0.001
Diabetes mellitus	0 (0.0)	0 (0.0)	···	3 (9.4)	4 (7.8)	1.000
Hyperlipidemia	0 (0.0)	1 (9.1)	1.000	6 (18.8)	8 (15.7)	0.717
Other cardiomyopathies	3 (50.0)	3 (27.3)	0.600	11 (34.4)	10 (19.6)	0.132
Atrial fibrillation	0 (0.0)	0 (0.0)	···	11 (34.4)	4 (7.8)	0.002
Echocardiography						
LVEDD, mm	59.0 (50.0–69.8)	47.0 (37.0–69.0)	0.350	64.5 (52.3–70.0)	61.0 (54.8–70.0)	0.581
LAD, mm	42.0 (30.3–50.5)	33.0 (27.0–36.0)	0.078	43.5 (36.8–50.0)	40.0 (34.0–46.5)	0.098
LVEF, %	34.0 (20.3–61.3)	61.0 (25.0–69.0)	0.180	31.6 (25.5–44.8)	40.0 (33.0–56.8)	0.016

Data are given as median (interquartile range) unless otherwise indicated. G+ indicates genotype positive; G−, genotype negative; LAD, left atrial diameter; LVEDD, left ventricular end-diastolic dimension; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association.

of which 27 (27.0%) experienced death and 5 (5.0%) underwent HT. All deaths were attributable to cardiovascular causes, consisting of 20 HF-related deaths, 6 SCDs, and 1 death attributable to another cardiovascular cause (Table 2).

Of the 17 children, 4 (23.5%) reached the primary end point, of whom 2 (50.0%) were G+ (Table S6). There was no significant difference in terms of the incidence of primary or secondary outcomes between G+ and G− patients (Table 2). Figure 2A displays the event-free survival curve, demonstrating no difference in the risk of the primary end point.

For the 83 adults, 28 (33.7%) experienced the primary end point. There was no significant difference in the incidence of the primary or secondary end points between children and adults (*P*=0.411, Table S7). G+ status was significantly more common in patients who reached the primary end point (57.1%, 16/28) than in those who did not (29.1%, 16/55; *P*=0.013; Table S8). Significantly more cases of the primary end point, HT, and HF-related death occurred in G+ patients than in G− patients (Table 2). No difference in incidence was observed between G+ and G− patients for the other end points. Multivariable Cox regression analyses showed that G+ status and New York Heart Association class III/IV at baseline were independently associated with an increased risk of the primary end point (hazard ratio, 2.49; 95% confidence interval, 1.15–5.37; *P*=0.020; and hazard ratio, 2.62; 95% confidence interval, 1.13–6.08; *P*=0.025, respectively) (Table 3). The event-free survival curve is displayed in Figure 2B. In addition, G+ status was associated with an increased risk of HF-related death in univariable analyses (Table 4). After adjustment, however, this association was no longer significant. No association between G+ status and other end points was observed.

Effect of Multiple Variants

Because only 1 of the 17 children carried >1 variant, the effect of multiple variants on phenotype was analyzed only in adults, of whom 6 were carriers of multiple variants. All of these 6 patients carried at least 1 variant in sarcomere genes. There was no significant difference in baseline characteristics or incidence of end points between carriers of multiple variants and single variant, except for a younger age at onset observed in the latter (Table 5). Univariable and multivariable Cox regression analyses showed that the risk of primary end point was significantly higher in carriers of single variant than noncarriers (hazard ratio, 2.45; 95% confidence interval, 1.08–5.54; *P*=0.032; Table 6). No other significant association was observed among carriers of multiple variants, single variant, and noncarriers.

Table 2. Incidence of Primary and Secondary End Points in G+ and G− Patients

End Point	All Patients (n=100)	Child Patients			Adult Patients		
		G+ (n=6)	G− (n=11)	*P* Value	G+ (n=32)	G− (n=51)	*P* Value
Primary							
Death and heart transplantation	32 (32.0)	2 (33.3)	2 (18.2)	0.584	16 (50.0)	12 (23.5)	0.013
Secondary							
All-cause death	27 (27.0)	1 (16.7)	2 (18.2)	1.000	12 (37.5)	12 (23.5)	0.172
Heart transplantation	5 (5.0)	1 (16.7)	0 (0.0)	0.353	4 (12.5)	0 (0.0)	0.020
Cardiovascular death	27 (27.0)	1 (16.7)	2 (18.2)	1.000	12 (37.5)	12 (23.5)	0.172
Sudden cardiac death	6 (6.0)	0 (0.0)	2 (18.2)	0.515	1 (3.1)	3 (5.9)	1.000
Heart failure–related death	20 (20.0)	1 (16.7)	0 (0.0)	0.353	11 (34.4)	8 (15.7)	0.049

Data are given as number (percentage). G+ indicates genotype positive; G−, genotype negative.

Discussion

In a large cohort of Chinese patients with LVNC, the genetic profile and correlations among genetics, clinical presentation, and long-term outcomes were comprehensively investigated. We found that 38% of the patients carried at least one pathogenic variant, with *TTN*, *MYH7*, *MYBPC3*, and *DSP* being the most commonly involved genes. The genetic spectrum was similar between children and adults. In adults, G+ status was associated with a higher risk of death and HT, independent of age, sex, and cardiac function at baseline.

With the development of next-generation sequencing, at least 21 genes, including sarcomeric, cytoskeletal, and ion channel genes, have been associated with LNVC.[4] Genetic testing may have implications in diagnosis and family screening, so it is recommended for patients with LVNC.[19] In accordance with a previously described yield of 35% to 40%,[4] the overall yield of our gene panel was 38%, despite differences in patient selection and gene panel used. The yield of testing was found to be higher in children than that in adults with LVNC,[12,20] whereas it was similar between children and adults in our study. This might be explained by the rather small numbers of child patients in our study.

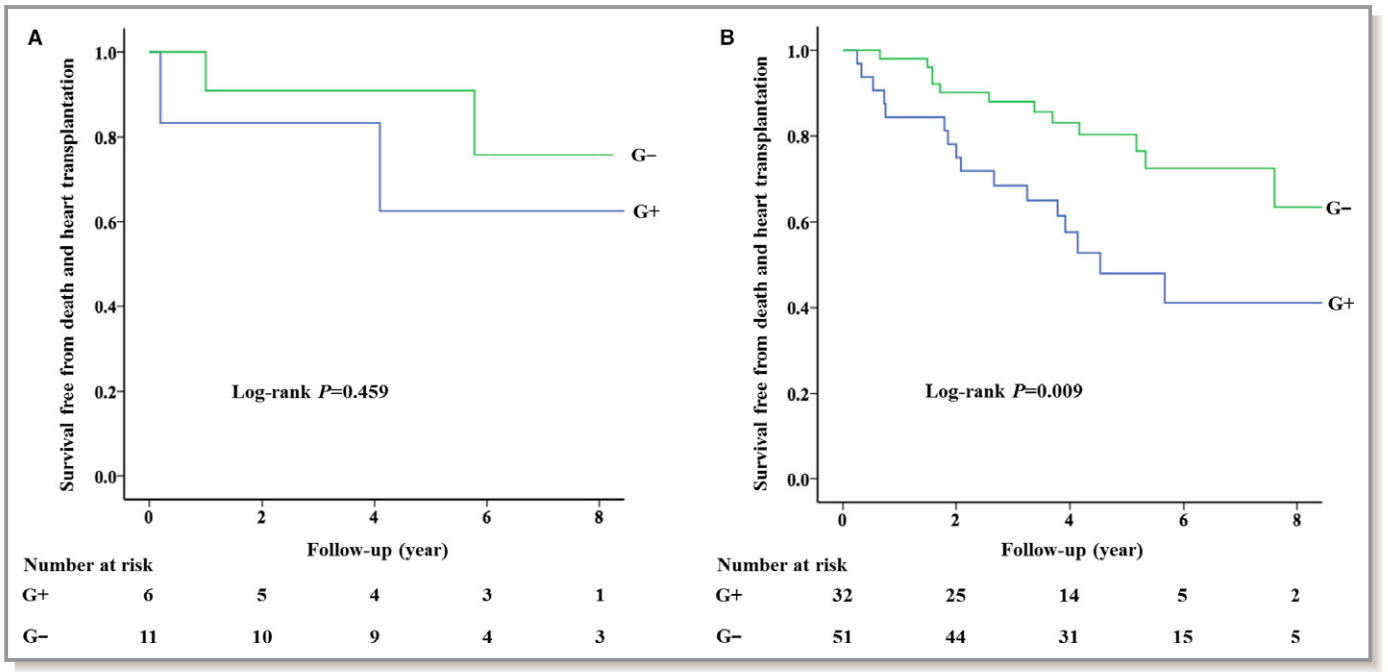

Figure 2. Survival curves free from death and heart transplantation in child patients (A) and adult patients (B) with left ventricular noncompaction cardiomyopathy. G+ indicates genotype positive; G−, genotype negative.

Table 3. Multivariable Analysis of Predictors of Primary End Points

	Univariable			Multivariable		
Variable	HR	95% CI	*P* Value	HR	95% CI	*P* Value
G+ status	2.62	1.23–5.54	0.012	2.49	1.15–5.37	0.020
Male sex	1.09	0.48–2.47	0.841	1.14	0.50–2.59	0.756
Age	1.01	0.99–1.03	0.461	1.01	0.98–1.04	0.629
NYHA class III/IV	2.93	1.29–6.68	0.010	2.62	1.13–6.08	0.025

CI indicates confidence interval; G+, genotype positive; HR, hazard ratio; NYHA, New York Heart Association.

Our study revealed a distinct genetic spectrum in Chinese Han patients with LVNC. *TTN*, *MYH7*, *MYBPC3*, and *DSP* were the most commonly involved genes, which accounted for >60% of all detected pathogenic variants. Despite studies being performed on different ethnicities, pathogenic variants in *MYH7* and *MYBPC3* were frequently detected in patients with LVNC and hypertrophic cardiomyopathy,[20–22] which indicated the shared genetic basis between different types of cardiomyopathy. Notably, variants in *DSP* were relatively common in our study. *DSP* encodes a critical component of desmosome structure in the myocardium and is a well-established causal gene of arrhythmogenic cardiomyopathy.[23,24] A truncating variant in *DSP* has been implicated in LVNC.[25] Besides, another truncating variant in *DSP* was found to be associated with SCD in a family.[26] In our study, 3 novel truncating variants in *DSP* were identified in 6 patients. Unexpectedly, ventricular arrythmias were observed in all of these 6 patients, suggesting a possible genotype-phenotype correlation. Therefore, patients with LVNC with pathogenic variants in *DSP* may have a high risk of arrhythmic events and should be followed up closely.

Two novel truncating variants in *LAMP2* were identified in 2 patients in our study. Deficiency in *LAMP2* has been shown to cause an X-linked lysosomal condition called Danon disease.[27] The role of *LAMP2* in LVNC was only described in a case report, in which a truncating *LAMP2* variant was described as having been detected in a patient with LVNC and Danon disease.[28] Herein, we provide additional evidence of the association between *LAMP2* deficiency and LVNC.

It is well recognized that 15% to 30% of patients with LVNC could experience premature death, and ≈10% could develop severe HF that may eventually lead to HT.[3,29] In our study, 27% of the patients died and 5% underwent HT, which also revealed the considerable morbidity and mortality in the process of LVNC. Despite the relatively poor prognosis, the effect of a positive genetic test result on long-term outcomes remains unclear in adult patients. Probst et al found that pathogenic variants were not associated with cardiac function, HF, or arrythmia events.[30] Similarly, a recent study by van Waning et al showed no difference in the risk of major adverse cardiac events between adults with LVNC with and without pathogenic variants.[20] In contrast, our study found that G+ status was independently associated with higher risks of death and HT in adult patients with LVNC. More than 70 sarcomere and nonsarcomere genes were screened in our study, whereas the study by Probst et al focused mainly on sarcomere genes.[30] The end point in the study by van Waning et al differed from ours, with thromboembolism and arrythmia events additionally being included.[20] Besides, only 6% of participants died and 2% received HT in their study, which were much less than those in our study. Such differences in screening genes and end points might at least partially explain the difference in results between these studies and ours. Our discovery is of great importance, because prognostic implications can be speculated from genetic testing. Such information can aid in risk stratification, which can then assist with therapeutic decision making and improve prognosis.

Table 4. Univariable and Multivariable Analyses of G+ Status and Secondary End Points

	Univariable			Multivariable*		
Variable	HR	95% CI	*P* Value	HR	95% CI	*P* Value
All-cause death	1.97	0.88–4.39	0.099	…	…	…
Heart transplantation	NA†	NA	NA	…	…	…
Cardiovascular death	1.97	0.88–4.39	0.099	…	…	…
Sudden cardiac death	0.62	0.06–5.92	0.674	…	…	…
Heart failure–related death	2.77	1.11–6.90	0.029	2.37	0.88–6.37	0.086

CI indicates confidence interval; G+, genotype positive; HR, hazard ratio; NA, not applicable.
*Items with $P<0.05$ in univariable analyses were then included in the calculation for multivariable HR and 95% CI.
†No heart transplantation occurred in genotype-negative patients, so HR and 95% CI are not available.

Table 5. Baseline Characteristics of Carriers of Multiple Variants and a Single Variant Among Adult Patients

Characteristic	Adult Patients (n=83)		
	Single Variant (n=26)	Multiple Variants (n=6)	*P* Value
Age at enrollment, y	42.5 (35.0–46.5)	55.0 (39.8–63.3)	0.069
Age at onset, y	38.5 (29.5–44.0)	52.5 (39.5–61.3)	0.033
Male sex, n (%)	18 (69.2)	4 (66.7)	1.000
Family history of cardiomyopathy, n (%)	8 (30.8)	1 (16.7)	0.648
NYHA class III/IV, n (%)	14 (53.8)	3 (50.0)	1.000
Comorbidities, n (%)			
Coronary artery disease	1 (3.8)	0 (0.0)	1.000
Hypertension	0 (0.0)	0 (0.0)	
Diabetes mellitus	2 (7.7)	1 (16.7)	0.476
Hyperlipidemia	4 (15.4)	2 (33.3)	0.310
Other cardiomyopathies	9 (34.6)	2 (33.3)	1.000
Atrial fibrillation	9 (34.6)	2 (33.3)	1.000
Echocardiography			
LVEDD, mm	66.5 (56.3–72.0)	58.5 (47.8–63.5)	0.119
LAD, mm	43.5 (38.0–50.3)	45.0 (35.3–50.3)	0.981
LVEF, %	30.5 (24.8–41.8)	41.5 (34.8–50.8)	0.087
Primary end point			
Death and heart transplantation	13 (50.0)	3 (50.0)	1.000
Secondary end point			
All-cause death	9 (34.6)	3 (50.0)	0.647
Sudden cardiac death	1 (3.8)	0 (0.0)	1.000
Heart failure–related death	8 (30.8)	3 (50.0)	0.390
Cardiovascular death	9 (34.6)	3 (50.0)	0.647
Heart transplantation	4 (15.4)	0 (0.0)	0.566

Data are given as median (interquartile range) unless otherwise indicated. LAD indicates left atrial diameter; LVEDD, left ventricular end-diastolic dimension; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association.

The specific mechanism underlying the adverse outcomes of G+ patients is still unclear. A plausible one is the remarkably abnormal structure and/or function of cardiomyocytes caused by the variants. For example, a defect in sarcomere genes can lead to the development of diminished force generation and myocardial fibrosis.[13,31] These changes may eventually result in contractile dysfunction and, therefore, may be associated with higher rates of HF events. In support, of all the adult patients with variants in sarcomere genes in our study, more than half had left ventricular ejection fraction <40% at baseline and one third died of HF during the follow-up. Defects in genes encoding ion channels, such as *KCNE1* and *SCN5A*, have been related with disturbed currents in cardiomyocytes, which may be associated with higher risk of arrhythmic events, including SCD.[32,33]

Multiple pathogenic variants have been associated with more severe manifestations and worse outcomes in patients with LVNC, compared with a single variant.[31,34] In our study, the risk of death and HT in carriers of multiple variants was higher but not significantly than that in carriers of a single variant or noncarriers. Because there were only 6 adult patients carrying >1 pathogenic variant, our failure to confirm a dosage effect of pathogenic variants might have been attributable to the lack of a sufficient number of participants for the results to reach a significant level.

There were several limitations in this study. First, this was an observational study, which might have been associated with an intrinsic bias. Second, all participants were from a single tertiary center. This might have caused a lack of representativeness and limited the generalizability of the findings. Third, the sample size was relatively small, so certain

Table 6. Univariable and Multivariable Analyses of Association Between Number of Detected Variants and Clinical Outcomes

	Univariable			Multivariable*		
Variable	HR	95% CI	*P* Value	HR	95% CI	*P* Value
Multiple vs single variant						
Death and heart transplantation	1.27	0.36–4.47	0.710	…	…	…
All-cause death	0.89	0.24–3.29	0.860	…	…	…
Heart transplantation	NA†	NA	NA	…	…	…
Cardiovascular death	0.89	0.24–3.29	0.860	…	…	…
Sudden cardiac death	NA‡	NA	NA	…	…	…
Heart failure–related death	0.80	0.21–3.04	0.748	…	…	…
Single vs no variant						
Death and heart transplantation	2.70	1.23–5.94	0.013	2.45	1.08–5.54	0.032
All-cause death	1.87	0.79–4.46	0.156	…	…	…
Heart transplantation	NA	NA	NA	…	…	…
Cardiovascular death	1.87	0.79–4.46	0.156	…	…	…
Sudden cardiac death	0.78	0.08–7.55	0.833	…	…	…
Heart failure–related death	2.56	0.96–6.86	0.061	…	…	…
Multiple vs no variant						
Death and heart transplantation	2.34	0.65–8.41	0.191	…	…	…
All-cause death	2.34	0.65–8.41	0.191	…	…	…
Heart transplantation	NA	NA	NA	…	…	…
Cardiovascular death	2.34	0.65–8.41	0.191	…	…	…
Sudden cardiac death	NA	NA	NA	…	…	…
Heart failure–related death	3.73	0.96–14.45	0.057	…	…	…

CI indicates confidence interval; HR, hazard ratio; NA, not applicable.
*Items with $P<0.05$ in univariable analyses were then included in the calculation of multivariable HR and 95% CI.
†No heart transplantation occurred in multiple variant carriers or noncarriers, so HR and 95% CI are not available.
‡No sudden cardiac death occurred in multiple variant carriers, so HR and 95% CI that refer to this group are not available.

conclusions could not be drawn in some subgroups, such as child patients. Studies in larger populations are needed to confirm our discoveries.

To our knowledge, this is one of the largest LVNC cohorts with detailed genetic information and long-term follow-up data in a Chinese Han population. We proved that, in adult patients with LVNC, G+ status was an independent risk factor for death and HT, highlighting the importance of genetic testing for better risk stratification of the individual patient.

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Disclosures

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SUPPLEMENTAL MATERIAL

Data S1.

Gene panel

We designed a panel of 72 gene which have been implicated in LVNC, other cardiomyopathies or ion channel disease. The panel consists of the following genes: *ABCC9, ACTC1, ACTN2, BAG3, CALR3, CASQ2, CAV3, CFL1, CFL2, CMYA5, CRIP2, CRYAB, DES, DMD, DMPK, DSC2, DSG2, DSP, DTNA, FHL1, FHL2, FLNC, GPD1L, HCN4, JPH2, JUP, KCNE1, KCNE2, KCNE3, KCNH2, KCNJ2, KCNQ1, LAMP2, LDB3, LMNA, MIB1, MYBPC3, MYH6, MYH7, MYL2, MYLK3, MYOM1, MYOM2, MYOZ1, MYOZ2, MYPN, NEXN, NNT, OBSCN, PDLIM3, PKP2, PLEC, PLN, PRDM16, PRKAG2, RBM20, RYR2, SCN5A, SGCD, SGCG, SLC25A4, SNTA1, TAZ, TCAP, TMEM43, TNNC1, TNNI3, TNNT2, TPM1, TTN, TTR, VCL.*

Table S1. Criteria for classifying pathogenic variants according to ACMG guideline

Evidence of pathogenicity		Category
Very strong	PVS1	null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where loss-of-function is a known mechanism of disease
Strong	PS1	Same amino acid change as a previously established pathogenic variant regardless of nucleotide change
	PS2	De novo (both maternity and paternity confirmed) in a patient with the disease and no family history
	PS3	Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product
	PS4	The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls
Moderate	PM1	Located in a mutational hot spot and/or critical and well-established functional domain without benign variation
	PM2	Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium
	PM3	For recessive disorders, detected in trans with a pathogenic variant
	PM4	Protein length changes as a result of in-frame deletions/insertions in a nonrepeat region or stop-loss variants
	PM5	Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before
	PM6	Assumed de novo, but without confirmation of paternity and maternity
Supporting	PP1	Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease
	PP2	Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease
	PP3	Multiple lines of computational evidence support a deleterious effect on the gene or gene product
	PP4	Patient’s phenotype or family history is highly specific for a disease with a single genetic etiology
	PP5	Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation

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PVS, very strong; PS, strong; PM, moderate; PP, supporting.

Table S2. Rules for combining criteria for pathogenic and likely pathogenic variants

Pathogenic	(i) 1 Very strong (PVS1) AND (a) ≥1 Strong (PS1–PS4) OR (b) ≥2 Moderate (PM1–PM6) OR (c) 1 Moderate (PM1–PM6) and 1 supporting (PP1–PP5) OR (d) ≥2 Supporting (PP1–PP5)
	(ii) ≥2 Strong (PS1–PS4)
	(iii) 1 Strong (PS1–PS4) AND (a)≥3 Moderate (PM1–PM6) OR (b)2 Moderate (PM1–PM6) AND ≥2 Supporting (PP1–PP5) OR (c)1 Moderate (PM1–PM6) AND≥4 supporting (PP1–PP5)
Likely pathogenic	(i) 1 Very strong (PVS1) AND 1 moderate (PM1–PM6)
	(ii) 1 Strong (PS1–PS4) AND 1–2 moderate (PM1–PM6)
	(iii) 1 Strong (PS1–PS4) AND ≥2 supporting (PP1–PP5)
	(iv) ≥3 Moderate (PM1–PM6)
	(v) 2 Moderate (PM1–PM6) AND ≥2 supporting (PP1–PP5)
	(vi) 1 Moderate (PM1–PM6) AND ≥4 supporting (PP1–PP5)

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PVS, very strong; PS, strong; PM, moderate; PP, supporting.

Table S3. Primers used for Sanger sequencing confirmation

	Forward primer	Reverse primer
ACTC1-1	ACTTAATTGATTTCTTACCGT	GTGTCAACTCAGGGTTAAATG
ACTC1-2	TACGGCCAGAAGCATACAGGG	CTTGACTTGGGCAGTTAGATA
DMD	ATATTTATGGGGTTATTACTA	CGAGCAGGGTCCAATTGTATC
DSC2	GCAACCTTGCATCTAGCCATA	GGCCTCATTTCAACATTGTTC
DSP-1	CCCGGACCTGCGCTACGAGGT	CGGGAAGTTCTTTCGGGACCT
DSP-2	CATAGATTTGCAACCTTGCCA	CTGGAGCCCCTTCAGGTATGC
DSP-3	ACAGAACGCTCCCGATATCAG	GCAGATGCTCCAGCGATAGAT
LAMP2-1	GCCATTACGAGCTTGTTATGC	GAGGGACACAGCAATATCAAA
LAMP2-2	TGTTCCGGTTGCAGAGTATAT	TATGCCCTTTAAAATGATAAT
LMNA	CCGAGATCGCGCCACTACACT	AAACAAACAGAAGCGCCACAA
MYBPC3-1	ATGTGCAGCACCTCAACTGGC	ACCTCCAGTGGGGGGCTCTGC
MYBPC3-2	CAGGATCCATTCGGCATTATA	GCTAACAGGATCCCGAAACTC
MYBPC3-3	CTGGAATGGGAGTGGGTTCAA	GGATTACAGGCGTCTGGCCTT
MYBPC3-4	ACATTATATTCTTTCGAGGAG	TGGTGCTCAGGCAATTATGTA
MYH7-2	GGGTCCCAACTCACATCGAAG	AGTGGGCAATGAGTACGTCAC
MYH7-3	TCTCTGTCCACCCAGGTGTAC	GGAAGGGACTCACTGGTAACT
MYH7-4	GAGAAAGACACCTAGCCATG	GACCGTCCGGAACGACAACTC
MYH7-5	GAGGAGGAATAGCAGTTGAAG	GACCAAGAACCCACCAATTCC
MYH7-6	GAGCAAGGTCAGCAAGGGTCC	CTCTTGCTGGGCTCCTTAATG
KCNE1-1	TCATGGGGAAGGCTTCGTCTC	AGCAGGGTGGCAACATGTCGG
KCNE1-2	GTGTGTTGGGTTGTTCTATGG	AGCTGCAGCAGTGGAACCTTA

NNT	AGAGAATGCTGGACATGTTCA	AAGAGAATGCTCAGTTTGACC
SCN5A	CCTGCCTCAGCCTTCCGAGTA	CCCCACTCCCTACAAGCTTTA
TNNT2	TTCCCAGTAATTATATCACAT	TGTCCTGACTTCTAACACCGT
TPM1	AGTCACAGGGGCAGGACTGAT	CCCCCACCCAGCAATATTAGA
TTN-1	GGCGTTCCACTTGTAGGTGA	GAGACTCCTGGAAAGGCCAC
TTN-2	GTTACTGGACCTGGCCTTCC	CCTGCTCCACCTAGGAGACT
TTN-3	ATTAACGGCCACAGACCGAG	GTGAACCAGTCCCTGCAAGA
TTN-4	AGGAGGTTGTGGCACTTCTG	TGTGAGAGTTCTGGACACGC
TTN-5	TGGAAGGGGTTTGCCAAGAA	CCAAGCCTACCATCAGAGCC
TTN-6	AAGGCAAGCTTGGTTCTCCA	AAAATAGGCACAGGGCCTCC
TTN-7	AGGTTTTCAGGCTCACCTGG	GGTCCGAGAAAAGAGGGTGG
TTN-8	AGGTTTTCAGGCTCACCTGG	GCACAGCACAATGGAACAGG
TTN-9	TACCGGCTGCATTGGAAACT	TTGAAAAGATCCCCCAGGGC
TTN-10	TGAAGGCTTGCTGACTCCTG	GTATTGGCCCACCTGTGGAA
TTN-11	TTTCAACAGGAGGGCCACAG	GGGGAGCTGGATAAAGACCG
TTN-12	AGACTGGGCCAAACATACCA	GAACCAGTTCAGGCCTCTCC
TTN-13	TGACAAAGGAGATGAGGTTGC	CTGCAGAGCCAGAAGTTCCA
TTN-14	CCAACAGGGCAGTAAGGGAA	AAGGGGTTGCTTCAGCTGTT
TTN-15	AGCATCTGAGGGGGAGATGT	TTGGATCCCAGGTTCCCCTA

Table S4. Pathogenic and likely pathogenic variants detected in the cohort.

Gene	dbSNP	Variant type	Transcript	Transcript effect	Protein effect	Novel variant	Pathogenicity*	Evidence†	gnomAD_ALL‡	gnomAD_EAS§	Carriers ID
ACTC1	rs193922680	missense	NM_005159	c.G301A	p.E101K		P	PS1,PS3,PM2,PP5	0.00000406	NA	90
	rs730880410	missense	NM_005159	c.T986C	p.I329T		LP	PS3,PM2	NA	NA	60
DMD		stop-gain	NM_000109	c.G7875A	p.W2625X	Novel	LP	PS3,PM2	NA	NA	43
		splicing	NM_000109	c.A3579+3T			LP	PS3,PM2	NA	NA	38
DSC2	rs193922708	missense	NM_004949	c.C835T	p.R279C		P	PS1,PS3	0.00004469	NA	9
DSP		frameshift insertion	NM_001008844	c.1_2insC	p.M1fs	Novel	LP	PVS1,PM2	NA	NA	72;99;114;115
		stop-gain	NM_001008844	c.C1138T	p.Q380X	Novel	LP	PVS1,PM2	NA	NA	66
		stop-gain	NM_001008844	c.G3901T	p.E1301X	Novel	LP	PVS1,PM2	NA	NA	70
KCNE1	rs79654911	missense	NM_000219	c.G200A	p.R67H		P	PS1,PS3,PM2,PP3,PP5	0.00005774	0.00005299	80
LAMP2		frameshift deletion	NM_001122606	c.371_375del	p.T124fs	Novel	LP	PVS1,PM2	NA	NA	13

		frameshift deletion	NM_001122606	c.325delT	p.Y109fs	Novel	LP	PVS1,PM2	NA	NA	17
LMNA		missense	NM_001257374	c.T998A	p.V333E		LP	PS3,PM2,PM5	NA	NA	40
MYBPC3	rs786204339	frameshift deletion	NM_000256	c.1377delC	p.P459fs	Novel	LP	PVS1,PM2	NA	NA	86
		frameshift deletion	NM_000256	c.1352_1379del	p.E451fs	Novel	LP	PVS1,PM2	NA	NA	104
		frameshift deletion	NM_000256	c.2568delG	p.R856fs	Novel	LP	PVS1,PM2	NA	NA	114
	rs397515887	missense	NM_000256	c.C1112T	p.P371L		LP	PS3,PM2	0.00002466	NA	43
MYH7	rs121913637	missense	NM_000257	c.C2155T	p.R719W		P	PS1,PS3,PM2,PP5	0.00003231	NA	105
	rs3218713	missense	NM_000257	c.G746A	p.R249Q		P	PS1,PS3,PM2,PP5	NA	NA	112
	rs730880161	missense	NM_000257	c.G2785A	p.E929K		P	PS1,PS3,PM2	NA	NA	48
	rs397516089	missense	NM_000257	c.G1106A	p.R369Q		P	PS1,PS3,PM2,PP5	NA	NA	64
	rs730880852	missense	NM_000257	c.C745G	p.R249G		P	PS1,PS3,PM2,PP5	NA	NA	51
NEXN	rs756273801	missense	NM_001172309	c.T488C	p.L163S		P	PS1,PS3	0.00001626	0.00017399	61

NNT		frameshift insertion	NM_012343	c.1770dupC	p.D590fs	Novel	LP	PVS1,PM2	NA	NA	105
SCN5A	rs45546039	missense	NM_000335	c.G665A	p.R222Q		P	PS1,PS3,PM2,PP3,PP5	NA	NA	72
	rs199473054	missense	NM_000335	c.G283A	p.V95I		P	PS1,PS3,PP3,PP5	0.00002886	0.00015898	27
TNNT2	rs121964856	missense	NM_000364	c.G305A	p.R102Q		P	PS1,PS3,PM2,PP3,PP5	NA	NA	9
TPM1	rs397516387	missense	NM_000366	c.C725T	p.A242V		LP	PS3,PM2	0.00000406	NA	41
TTN		frameshift insertion	NM_001256850	c.55176dupA	p.E18393fs	Novel	LP	PVS1,PM2	NA	NA	79
		frameshift deletion	NM_001256850	c.50906_50907del	p.P16969fs	Novel	LP	PVS1,PM2	NA	NA	81
		frameshift deletion	NM_001256850	c.8228delA	p.N2743fs	Novel	LP	PVS1,PM2	NA	NA	86
		splicing	NM_001256850	c.G13141+1A		Novel	LP	PVS1,PM2	NA	NA	92
		stop-gain	NM_001256850	c.C72093G	p.Y24031X	Novel	LP	PVS1,PM2	NA	NA	93
		stop-gain	NM_001256850	c.C56939A	p.S18980X	Novel	LP	PVS1,PM2	NA	NA	100

		frameshift deletion	NM_001256850	c.98135delA	p.H32712fs	Novel	LP	PVS1,PM2	NA	NA	102
		splicing	NM_001256850	c.40222+2insAATA		Novel	LP	PVS1,PM2	NA	NA	104
		stop-gain	NM_001256850	c.C80167T	p.R26723X	Novel	LP	PVS1,PM2	NA	NA	116
		frameshift deletion	NM_001256850	c.58109delT	p.V19370fs	Novel	LP	PVS1,PM2	NA	NA	117
		stop-gain	NM_001256850	c.C44607A	p.Y14869X	Novel	LP	PVS1,PM2	NA	NA	120
		stop-gain	NM_001256850	c.A88642T	p.K29548X	Novel	LP	PVS1,PM2	NA	NA	45
	rs779485172	splicing	NM_001256850	c.A63605-2T	.	Novel	LP	PVS1,PM2	NA	NA	29
		frameshift deletion	NM_001256850	c.56615_56618del	p.E18872fs	Novel	LP	PVS1,PM2	NA	NA	73
		frameshift insertion	NM_001256850	c.48473_48474insGCTTT	p.F16158fs	Novel	LP	PVS1,PM2	NA	NA	34

* Determined according to criteria in Table S2

† As listed in Table S1

‡ Minor allele frequencies of variants among total population in the Genome Aggregation Database (*Nature*. 2016;536: 285-91)

§ Minor allele frequencies of variants among East Asians in the Genome Aggregation Database (*Nature*. 2016;536: 285-91)

LP, likely pathogenic; NA, not available; P, pathogenic

Table S5. Clinical characteristics of the six carriers of *DSP* variant

Patient ID	Age at onset	Sex	NYHA class	Arrythmia	Enlarged RV	Antiarrhythmics	Other treatment	Outcome
66	20	Male	III	SUVT	No	Amiodarone		HT
70	44	Male	II	SUVT	No		RFCA	
72	17	Male	II	NSVT	No	Amiodarone	ICD	
99	40	Male	II	SUVT, AF	No	Amiodarone		
114	60	Male	III	SUVT	No	Amiodarone		HF-related death
115	51	Female	II	SUVT	No	Amiodarone	ICD	

AF, atrial fibrillation; HF, heart failure; HT, heart transplantation; ICD, implantable cardioverter defibrillator; NYHA, New York Heart Association; NSVT, non-sustained ventricular tachycardia; RFCA, radiofrequency catheter ablation; RV, right ventricle; SUVT, sustained ventricular tachycardia.

Table S6. Characteristics of child patients who reached the primary endpoint.

Patient ID	Sex	Age at onset	NYHA functional class	Family history	Outcome	Pathogenic variants
10	Male	16	I/II	No	SCD	
13	Male	16	III/IV	No	HF-related death	LAMP2 p.T124fs
64	Male	3	III/IV	No	Heart transplantation	MYH7 p.R369Q
84	Male	8	I/II	No	SCD	

HF indicates heart failure; NYHA, New York Heart Association; SCD, sudden cardiac death.

Table S7. Incidence of primary and secondary endpoints in child and adult patients.

Endpoints	Children (n=17)	Adult (n=83)	*P*-Value
Death and heart transplantation, n (%)	4 (23.5)	28 (33.7)	0.411
All-cause death, n (%)	3 (17.6)	24 (28.9)	0.549
Sudden cardiac death, n (%)	2 (11.8)	4 (4.8)	0.269
Heart failure-related death, n (%)	1 (5.9)	19 (16.6)	0.182
Cardiovascular death, n (%)	3 (17.6)	24 (28.9)	0.549
Heart transplantation, n (%)	1 (5.9)	4 (4.8)	1.000

Table S8. Characteristics of adult patients who reached the primary endpoint.

Patient ID	Sex	Age at onset	NYHA functional class	Family history	Outcome	Pathogenic variants
14	Male	42	III/IV	No	HF-related death	
15	Female	39	III/IV	No	HF-related death	
17	Female	19	III/IV	Yes	HF-related death	LAMP2 p.Y109fs
18	Male	44	III/IV	No	HF-related death	
19	Male	43	III/IV	No	HF-related death	
22	Female	70	III/IV	No	HF-related death	
27	Male	50	III/IV	No	HF-related death	SCN5A p.V95I
32	Male	52	III/IV	No	HF-related death	
34	Male	18	III/IV	No	HF-related death	TTN p.F16158fs
41	Male	44	III/IV	Yes	HF-related death	TPM1 p.A242V
43	Male	59	III/IV	No	HF-related death	DMD p.W2625X;MYBPC3 p.P371L
45	Male	39	III/IV	No	HF-related death	TTN p.K29548X
51	Female	31	III/IV	Yes	HF-related death	MYH7 p.R249G
55	Female	26	III/IV	No	HF-related death	
57	Male	34	I/II	No	SCD	
61	Male	39	I/II	Yes	SCD	NEXN p.L163S
66	Male	20	III/IV	No	Heart transplantation	DSP p.Q380X
67	Male	37	III/IV	No	HF-related death	
68	Female	52	I/II	No	SCD	

73	Male	78	I/II	No	HF-related death	TTN p.E18872fs; TTN p.G4397D
77	Male	66	I/II	No	Other cardiovascular death	
80	Male	40	III/IV	No	Heart transplantation	KCNE1 p.R67H
93	Male	28	I/II	No	Heart transplantation	TTN p.Y24031X
99	Male	40	I/II	No	HF-related death	DSP p.M1fs
102	Male	23	III/IV	No	Heart transplantation	TTN p.H32712fs
105	Female	46	I/II	No	HF-related death	MYH7 p.R719W; NNT p.D590fs
110	Female	41	III/IV	No	SCD	
114	Male	60	III/IV	No	HF-related death	DSP p.M1fs; MYBPC3 p.R856fs

HF indicates heart failure; NYHA, New York Heart Association; SCD, sudden cardiac death.