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Clinical implications of sarcomere and nonsarcomere gene variants in patients with left ventricular noncompaction cardiomyopathy

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Abstract

Background: Robust data regarding genotype–phenotype correlations in left ventricular noncompaction cardiomyopathy (LVNC) are lacking.

Methods: About 72 cardiomyopathy-related genes were comprehensively screened in a cohort of LVNC patients using targeted sequencing. Baseline and follow-up data were collected. The primary endpoint was a composite of death and heart transplantation.

Results: A total of 83 unrelated adult patients were included in analyses. Following stringent classification according to the American College of Medical Genetics and Genomics (ACMG) guidelines, 36 pathogenic variants of 14 genes were detected in 32 patients. Among them, 12 patients carried at least one nonsarcomere variant (NSV). At baseline, NSV carriers had a higher frequency of atrial fibrillation, but lower left ventricular ejection fraction, than did noncarriers. During a median follow-up of 4.2 years, NSV carriers experienced a higher rate of the primary endpoint compared with noncarriers. There was no significant difference in the rate between carriers of sarcomere variant (SV) and noncarriers, as well as between carriers of SV and NSV. The presence of NSV was associated with an increased risk of the primary endpoint independent of age, sex, and cardiac function (hazard ratio: 3.61, 95% confidence interval: 1.42–9.19, $p = .002$).

Conclusion: NSV may act as a genetic modifier and worsen the clinical phenotype in patients with LVNC.

KEYWORDS
genotype, left ventricular noncompaction cardiomyopathy, prognosis

1 | INTRODUCTION

Left ventricular noncompaction cardiomyopathy (LVNC), which is characterized by abnormal trabeculations in the left ventricle, is present in 3%–4% of patients with heart failure. (Kovacevic-Preradovic et al., 2009; Patrianakos, Parthenakis, Nyktari, & Vardas, 2008) Clinical presentations of adult patients with LVNC are highly heterogeneous, varying from no apparent symptoms to serious complications, such as heart failure, arrhythmia, or thromboembolism. (Finsterer, Stollberger, & Towbin, 2017) Therefore, identifying high-risk patients and providing them with proper treatment to improve prognosis are important.

Left ventricular noncompaction cardiomyopathy was classified as a genetic cardiomyopathy by the American Heart Association. (Maron et al., 2006) LVNC has been reported in association with >40 genes,(Finsterer et al., 2017) which can be broadly divided into sarcomere and nonsarcomere genes. Recent studies have shown that sarcomere variants (SV) account for most of the genetic defects in LVNC, with *MYH7*(OMIM 160760), *MYBPC3* (OMIM 600958), and *TTN* (OMIM 188840) as the most prevalent genes. (Sedaghat-Hamedani et al., 2017; van Waning et al., 2018).

A predictable association between genotype and phenotype is needed to use genetic testing for risk assessment. Most previous studies attempted to relate SV with disease expression in LVNC but yielded disappointing results. (Probst et al., 2011; Tian et al., 2015) However, whether there is a correlation between nonsarcomere variants (NSVs) and clinical outcomes remains unknown. To address this issue, we conducted the present study in a Chinese cohort of adult patients with LVNC.

2 | METHODS

2.1 | Ethical compliance

The study complied with the principles of the Declaration of Helsinki and was approved by the Ethics Committee of Fuwai Hospital. Written informed consent was obtained from all participants.

2.2 | Study design and participants

Data were obtained from a cohort study, which enrolled 100 unrelated patients with LVNC at Fuwai Hospital between April 2004 and May 2016. Patients enrollment, genetic sequencing, variant classification, and follow-up have been described in a previous study. (Li et al., 2018) Briefly, the diagnosis of LVNC was based on echocardiographic or cardiac magnetic resonance findings according to Jenni et al or Petersen et al criteria. (Jenni, Oechslin, Schneider, Attenhofer Jost, & Kaufmann, 2001; Petersen et al., 2005) Patients were eligible if they had LVNC and were willing to receive genetic testing and follow-up. Since there are significant differences in genetic predisposition between child and adult patients (van Waning et al., 2018), only adult patients were included in the current analyses. Outcome data were obtained through a telephone interview or clinic visit. The last follow-up was performed in April 2018.

2.3 | Targeted sequencing

After informed consent was acquired, peripheral venous blood was collected for genomic DNA extraction. The coding exons and their adjacent 10-bp intronic sequences of 72 cardiomyopathy-related genes were comprehensively screened using targeted resequencing. Details about the genes being tested are described in supplementary material. The mean depth of all samples was more than 400×, with coverage of more than 99.7%. Variants were described according to the guidelines for mutation nomenclature of the Human Genome Variation Society (http://www.hgvs.org/). Variants were excluded if their minor allele frequency was ≥0.05% among East Asians in the Genome Aggregation Database. (Lek et al., 2016) 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."(Richards et al., 2015) In the current analysis, variants that were classified as "pathogenic" or "likely pathogenic" were considered to be pathogenic and divided into SV or NSV. Accordingly, patients were grouped into carriers of SV, carriers of NSV, or noncarriers. Sanger sequencing was used to validate the presence of pathogenic variants.

2.4 | Study endpoints

The primary endpoint was a composite of death and heart transplantation (HT). The secondary endpoints included all-cause death, HT, and cardiovascular death. Cardiovascular death included sudden cardiac death (SCD), heart failure (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 hr 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 hr.

2.5 | Statistical analysis

Continuous variables are expressed as median (interquartile range) and categorical variables are presented as frequency and percentage. Analysis of variance was performed for comparison of continuous variables and Pearson chi-square test or Fisher's exact test was performed for comparison of category variables. Univariable or multivariable Cox proportional

hazards models were performed to calculate the hazard ratio (HR) and 95% confidence interval (CI) 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 that were 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 two-sided *p*-value was <.05. All analyses were performed with SPSS version 22.0 software (IBM Corp.).

3 | RESULTS

3.1 | Study population and genetic findings

A total of 83 unrelated adult patients were included in the current analyses. Among them, 32 (38.6%) patients had 36 pathogenic variants in 14 genes, including 20 carriers of SV and 12 carriers of NSV (Tables 1 and S1). *TTN*, *MYH7*, and *MYBPC3* were the most commonly involved sarcomere genes, while *DSP* (OMIM 125647) and *DMD* (OMIM 300377) were the most frequently mutated nonsarcomere genes.

3.2 | Baseline characteristics

Baseline characteristics are shown in Table 2. SV carriers had a higher prevalence of a family history of cardiomyopathy, atrial fibrillation, and atrioventricular block but a lower rate of hypertension, compared with noncarriers ($p < .05$ for all). NSV carriers had a higher frequency of atrial fibrillation, but lower left ventricular ejection fraction (LVEF), than did noncarriers ($p < .05$ for all). There were no significant differences in baseline characteristics between carriers of SV and NSV.

3.3 | Clinical outcomes

During a median follow-up of 4.2 years, 28 (33.7%) patients reached the primary endpoint, including 24 deaths and four HT (Table 3). Carriers of NSV experienced significantly higher rates of the primary endpoint, HT, and heart failure-related death compared with noncarriers ($p < .05$ for all). There were no significant differences in rates of primary or secondary endpoints between carriers of SV and noncarriers, as well as between carriers of SV and NSV.

TABLE 1 Demographic, genetic, and clinical findings in patients with pathogenic variant of nonsarcomere gene

Patient ID	Sex	Age at enrollment	Variant	Family history	Complicating cardiomyopathy/ myopathy	NYHA class at baseline	Arrythmia at baseline	Outcome
9	Female	44	*DSC2* c.C835T; *TNNT2* c.G305A	Sister: HCM	HCM	III	AF	
17	Female	20	*LAMP2* c.325delT	Mother: LVNC		IV	Ventricular preexcitation VT, AF, AVB	HF-related death
27	Male	53	*SCN5A* c.G283A	No		IV	PVC	HF-related death
38	Male	35	*DMD* c.A3579 + 3T	No	DMD	IV	PVC, VT	
43	Male	67	*DMD* c.G7875A; *MYBPC3* c.C1112T	No		III	PVC, VT	HF-related death
66	Male	24	*DSP* c.C1138T	No	DCM	III	VT	HT
70	Male	45	*DSP* c.G3901T	No		II	VT	
80	Male	43	*KCNE1* c.G200A	No	DCM	IV	PVC	HT
99	Male	42	*DSP* c.1_2insC	No		II	VT, AF	HF-related death
105	Female	49	*NNT* c.1770dupC; *MYH7* c.C2155T	No	HCM	II	AF, LBBB	HF-related death
114	Male	61	*DSP* c.1_2insC; *MYBPC3* c.2568delG	No		III	AF, PVC, VT	HF-related death
115	Female	53	*DSP* c.1_2insC	No		II	VT	

Abbreviations: AF, atrial fibrillation; AVB, atrioventricular block; LBBB, left bundle branch block; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; HF, heart failure; HT, heart transplantation; NYHA, New York Heart Association; DMD, Duchenne muscular dystrophy; PVC, premature ventricular contraction; VT, ventricular tachycardia.

TABLE 2 Baseline characteristics of nonsarcomere gene variants carriers and noncarriers

Characteristics	All patients ($n = 83$)	SV carriers ($n = 20$)	NSV carriers ($n = 12$)	Noncarriers ($n = 51$)	$p^{\dagger}$
Age at enrollment, year	44.0 (34.0–55.0)	43.0 (35.5–47.5)	44.5 (36.8–53.0)	46.0 (30.0–57.0)	NS
Age of onset, year	40.0 (28.0–51.0)	37.5 (28.5–43.5)	44.0 (33.3–50.8)	42.0 (28.0–52.0)	NS
Male, *n* (%)	58 (69.9)	14 (70.0)	8 (66.7)	36 (70.6)	NS
Family history of cardiomyopathy, *n* (%)	11 (13.3)	7 (35.0)	2 (16.7)	2 (3.9)	A;b
NYHA class III/IV, *n* (%)	39 (47.0)	9 (45.0)	8 (66.7)	22 (43.1)	NS
Comorbidities					
Coronary artery disease, *n* (%)	9 (10.8)	0 (0.0)	1 (8.3)	8 (15.7)	NS
Hypertension, *n* (%)	13 (15.7)	0 (0.0)	0 (0.0)	13 (25.5)	A;b
Diabetes, *n* (%)	7 (8.4)	1 (5.0)	2 (16.7)	4 (7.8)	NS
Hyperlipidemia, *n* (%)	14 (16.9)	4 (20.0)	2 (16.7)	8 (15.7)	NS
Other cardiomyopathies, *n* (%)	21 (25.3)	7 (35.0)	4 (33.3)	10 (19.6)	NS
Arrhythmia					
Atrial fibrillation, *n* (%)	15 (18.1)	7 (35.0)	4 (33.3)	4 (7.8)	A;b;c
Premature ventricular contraction	47 (56.6)	10 (50.0)	9 (75.0)	28 (54.9)	NS
Ventricular tachycardia	32 (38.6)	9 (45.0)	7 (58.3)	16 (31.4)	NS
Atrioventricular block	16 (19.3)	8 (40.0)	2 (16.7)	6 (11.8)	A;b
LBBB	20 (24.1)	5 (25.0)	1 (8.3)	14 (27.5)	NS
RBBB	3 (3.6)	2 (10.0)	0 (0.0)	1 (2.0)	NS
Echocardiography					
LVEDD, mm	62.0 (54.8–70.0)	64.5 (50.3–71.3)	65.5 (57.5–70.0)	61.0 (54.8–70.0)	NS
LAD, mm	41.5 (35.0–48.0)	42.5 (33.8–50.8)	44.5 (41.5–49.8)	40.0 (34.0–46.5)	NS
LVEF, %	38.5 (30.8–52.3)	33.1 (28.5–47.0)	29.0 (24.3–43.0)	40.0(33.0–56.8)	c
Treatment					
Pacemaker, *n* (%)	4 (4.8)	2 (10.0)	0 (0.0)	3 (5.9)	NS
CRT, *n* (%)	7 (8.4)	2 (10.0)	1 (8.3)	5 (9.8)	NS
ICD, *n* (%)	10 (12.0)	4 (20.0)	2 (16.7)	4 (7.8)	NS

Abbreviations: CRT, cardiac resynchronization therapy; ICD, implantable cardioverter defibrillator; LAD, left atrial diameter; LVEDD, left ventricular end-diastolic dimension; LVEF, left ventricular ejection fraction; LBBB, left bundle branch block; NYHA, New York Heart Association; RBBB, right bundle branch block.

†A, significant difference between three groups; a, significant difference between the SV Carriers group and NSV Carriers group; b, significant difference between the SV Carriers group and Noncarriers group; c, significant difference between the NSV Carriers group and Noncarriers group; NS, not significant.

Univariable analyses showed that NSV was associated with an increased risk of death and HT compared with no variant ($p = .002$, Table 4 and Figure 1). Multivariable analysis showed that NSV was an independent risk factor of death and HT (HR: 3.61, 95% CI: 1.42–9.19, $p = .007$, Table 4). For secondary endpoints, univariable analyses showed that NSV was associated with higher risks of all-cause death, cardiovascular death, and heart failure-related death compared with no variant (Table 4 and Figure 1). After adjustment, NSV remained to be associated with increased risks of all-cause death (HR: 2.88, 95% CI: 1.04–7.96, $p = .042$), cardiovascular death (HR: 2.88, 95% CI: 1.04–7.96, $p = .042$), and HF-related death (HR: 3.97, 95% CI: 1.28–12.24, $p = .017$, Table 4). There was no significant association between SV and primary or secondary endpoints (Table 4).

4 | DISCUSSION

Among 83 adult patients with LVNC, we found that 12 (14.5%) carried at least 1 NSV, and *DSP* and *DMD* were the most commonly involved nonsarcomere genes. At baseline, NSV carriers had a higher prevalence of atrial fibrillation and lower LVEF compared with noncarriers. During follow-up, the presence of NSV was independently associated with an increased risk of a composite of death and HT, while the presence of SV was not significantly associated with clinical outcomes.

As a common genetic defect in LVNC, SV is identified in approximately 20%–30% of patients. (Probst et al., 2011; Sedaghat-Hamedani et al., 2017; van Waning et al., 2018) There was no significant association between SV and clinical outcomes in previous studies (Table 5) and in our study. Aside from SV, NSV are also found in a minor proportion of patients

TABLE 3 Incidence of primary and secondary endpoints

	All patients ($n = 83$)	SV carriers ($n = 20$)	NSV carriers ($n = 12$)	Noncarriers ($n = 51$)	p
Primary endpoint					
Death and heart transplantation, *n* (%)	28 (33.7)	8 (40.0)	8 (66.7)	12 (23.5)	A; c
Secondary endpoints					
All-cause death, *n* (%)	24 (28.9)	6 (30.0)	6 (50.0)	12 (23.5)	NS
Heart transplantation, *n* (%)	4 (4.8)	2 (10.0)	2 (16.7)	0 (0.0)	A; c
Cardiovascular death, *n* (%)	24 (28.9)	6 (30.0)	6 (50.0)	12 (23.5)	NS
Sudden cardiac death, *n* (%)	4 (4.8)	1 (5.0)	0 (0.0)	3 (5.9)	NS
Heart failure-related death, *n* (%)	19 (22.9)	5 (25.0)	6 (50.0)	8 (15.7)	A; c

Note: p values: A, significant difference between three groups; c, significant difference between the NSV Carriers group and Non-carriers group; NS, not significant.

TABLE 4 Univariable and multivariable analyses of association between detected variants and clinical outcomes

	Univariable			Multivariable[†]		
	HR	95% CI	p	HR	95% CI	p
SV versus no variant						
Death and heart transplantation	1.95	0.80–4.79	.143	–	–	–
All-cause death	1.46	0.55–3.90	.450	–	–	–
Heart transplantation	NA[‡]	NA[‡]	NA[‡]	–	–	–
Cardiovascular death	1.46	0.55–3.90	.450	–	–	–
Sudden cardiac death	0.92	0.10–8.89	.945	–	–	–
Heart failure-related Death	1.88	0.61–5.75	.271	–	–	–
NSV versus no variant						
Death and heart transplantation	4.05	1.64–9.98	.002	3.61	1.42–9.19	.007
All-cause death	3.09	1.15–8.30	.025	2.88	1.04–7.96	.042
Heart transplantation	NA[‡]	NA[‡]	NA[‡]	–	–	–
Cardiovascular death	3.09	1.15–8.30	.025	2.88	1.04–7.96	.042
Sudden cardiac death	NA[§]	NA[§]	NA[§]	–	–	–
Heart failure-related Death	4.80	1.65–13.99	.004	3.97	1.28–12.24	.017

Abbreviations: CI, confidence interval; HR, hazard ratio.

[†]Items with $p < .05$ in univariable analyses were then included in the calculation of multivariable HR and 95% CI.

[‡]No heart transplantation occurred in noncarriers, so HR and 95% CI are not available.

[§]No sudden cardiac death occurred in NSV carriers, so HR and 95% CI are not available.

with LVNC, including variants in cytoskeletal, ion channel, and mitochondrial genes. (Finsterer et al., 2017) In contrast to a recent study by van Waning et al. (Table 5), our study showed that NSV could increase the risk of adverse events in adult patients with LVNC. There were some differences between these studies that should be noted. Our study focused only on adult patients while in the study by van Waning et al, the multivariable analysis regarding NSV was performed among patients of all ages. Besides, the primary endpoint in van Waning et al study differed from ours, with thromboembolism and arrythmia events additionally being included. These differences might contribute to the contradictory results between two studies.

In our previous study (Li et al., 2018), the presence of pathogenic variants (SV/NSV) was found to be associated with an increased risk of death and HT among adult patients with LVNC. Further analyses in the current study showed that the majority of this increased risk was accounted by the presence of NSV rather than SV. Therefore, patients with pathogenic variants, especially those with NSV, may have a high risk of adverse events and should be followed up closely.

Loss-of-function variants in *SCN5A* (OMIM 600163), which encodes the α-subunit of the cardiac sodium channel, can cause dilated cardiomyopathy, Brugada syndrome, cardiac conduction defect, sick sinus syndrome, and atrial fibrillation. (Wilde & Amin, 2018) Although the relationship

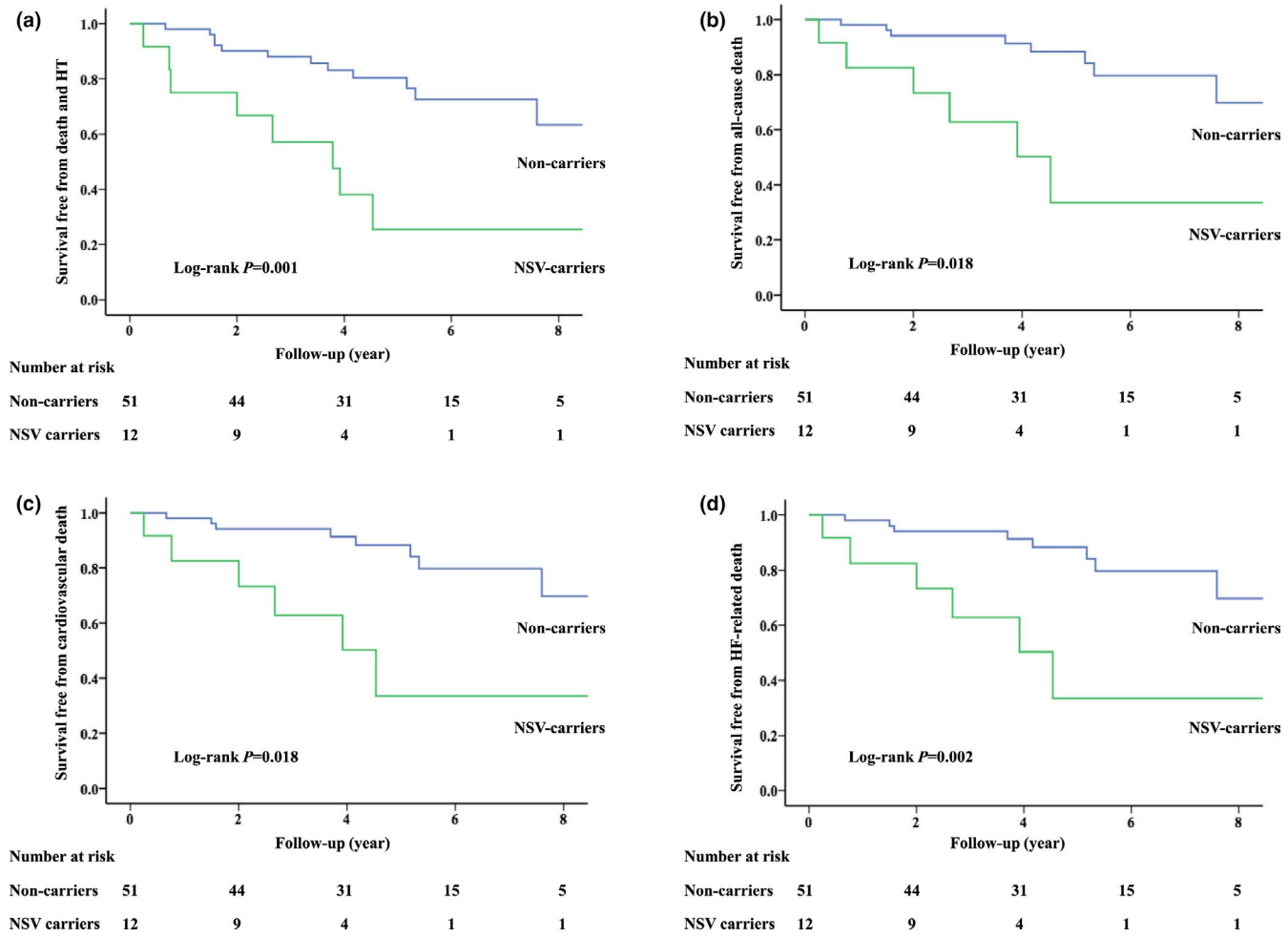

FIGURE 1 Survival curves free from death and heart transplantation (a), all-cause death (b), cardiovascular death (c), and heart failure-related death (d) in adult patients. Abbreviations: HF, heart failure; HT, heart transplantation; NSV, nonsarcomere variant

between *SCN5A* and LVNC remains to be established, the presence of *SCN5A* variant increases the risk of arrhythmia and HF in LVNC patients. (Shan et al., 2008) There is no association between *KCNE1* (OMIM 176261) and changes in left ventricular morphology, but *KCNE1* variant has an effect on rectifier K^+ current and has been recognized as a cause for long QT syndrome. (Nishio et al., 2009) Therefore, variants in *SCN5A* and *KCNE1* can lead to increased susceptibility to arrhythmia and result in an adverse prognosis.

DSP and *DSC2* (OMIM 125645) encode desmosome proteins and are established disease genes for arrhythmogenic right ventricular cardiomyopathy. (Corrado, Basso, & Judge, 2017) A truncating variant in *DSP* is also related to LVNC and severe early-onset HF. (Williams et al., 2011) An association between *DSC2* and LVNC has not been reported to date. Knockdown of *DSC2* in zebrafish embryos can cause myocardial contractility defects, (Heuser et al., 2006) suggesting that *DSC2* variants may be able to impair contractile function and eventually lead to HF.

Defects in *DMD* and *NNT* (OMIM 607878) have been associated with LVNC. *DMD* encodes the structural cytoskeletal protein dystrophin. Mutations in *DMD* result in Duchenne/Becker muscular dystrophy. (Kamdar & Garry, 2016) Patients with LVNC have an increased risk of mortality when complicated by neuromuscular disorders. (Stollberger et al., 2015; Stollberger, Blazek, Wegner, Winkler-Dworak, & Finsterer, 2011) *NNT* encodes a mitochondrial protein. Suppression of *Nnt* in zebrafish causes early ventricular malformation and contractility defects,(Bainbridge et al., 2015) suggesting that a defect in *NNT* may contribute to susceptibility to HF.

LAMP2 (OMIM 309060) cardiomyopathy is a profound disease process that is characterized by progressive HF and severe arrhythmia, rapidly leading to cardiac death in young patients. (Maron et al., 2009) The clinical features of this disease include left ventricular hypertrophy, atrial fibrillation, and ventricular preexcitation. LVNC has also been reported as a phenotype of *LAMP2* cardiomyopathy. (Van Der Starre et al., 2013) In our study, one female patient with the *LAMP2* variant presented with LVNC, atrial fibrillation, and ventricular preexcitation, but without apparent ventricular hypertrophy, and died of progressive HF at a young age. These findings provide additional evidence for the adverse prognosis and clinical heterogeneity of *LAMP2* cardiomyopathy.

TABLE 5 An overview of previous studies on the relationship between SV/NSV and phenotype in LVNC patients

Study	Study population	Genetic testing	Prevalence	Relationship with phenotype
Sarcomere variants				
Probst et al., 2011	63 European probands (adults and children)	8 genes	18/63 (29%) with mutated *MYH7*, *MYBPC3*, *ACTC1*, *TNNT2, or TPM1*	No significant differences in average age, cardiac function, and heart failure or tachyarrhythmias at baseline or follow-up between carriers and noncarriers
Tian et al., 2015	57 Chinese probands (adults and children)	10 genes	7/57 (12%) with mutated *MYH7*, *ACTC1*, *TNNT2, or TPM1*	No significant differences in clinical characteristics at baseline and mortality during follow-up between carriers and noncarriers
van Waning et al., 2018	327 European probands (adults and children)	45 genes	85/327 (26%) with mutated *MYH7*, *MYBPC3*, *TTN*, *ACTC1*, *ACTN2*, *TNNC1, TNNT2*, *MYL2*, *or TPM1*	No significant differences in adverse events at baseline or follow-up between carriers and noncarriers
Nonsarcomere variants				
van Waning et al., 2018	327 European probands (adults and children)	45 genes	19/327 (6%) with mutated *DES*, *DSP*, *FKTN*, *HCN4*, *KCNQ1*, *LAMP2*, *LMNA*, *MIB1*, *NOTCH1*, *PLN*, *RYR2*, *SCN5A*, or *TAZ*	No significant differences in adverse events at baseline or follow-up between carriers and noncarriers

Although most of these nonsarcomere genes have no clear relevance with LVNC, the presence of pathogenic variants in these genes was associated with serious manifestations in patients with LVNC in our study, including a lower LVEF and an increased risk of adverse outcomes. This finding suggests that NSV, at least some of them, may act as genetic modifiers and influence phenotypic expression, which could partially explain the remarkable heterogeneity in the clinical manifestation of LVNC. However, additional studies are required to replicate and further evaluate the potential effect of NSVs on LVNC.

There are some limitations that should be noted in this study. First, this was an observational study and all of the patients were recruited from a specialized center for cardiovascular diseases, which might have led to selection and measurement bias. Second, the sample size was relatively small, which might have limited the power of our findings. Third, despite the fact that more than 70 cardiomyopathy-associated genes were comprehensively screened, some other nonsarcomere genes might also have an effect on the disease, which could not be evaluated.

To the best of our knowledge, this is the first study reporting that the presence of NSV is associated with adverse outcomes in adult patients with LVNC. NSV, at least some of them, may act as genetic modifiers and influence the clinical phenotype. These findings may aid in risk stratification in patients with LVNC.

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CONFLICT OF INTEREST

None.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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