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ORIGINAL RESEARCH

HEART FAILURE

Association of Multiple Nonhypertrophic Cardiomyopathy–Related Genetic Variants and Outcomes in Patients With Hypertrophic Cardiomyopathy

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ABSTRACT

BACKGROUND Approximately 10% of hypertrophic cardiomyopathy (HCM) patients have left ventricular systolic dysfunction (end-stage HCM) leading to severe heart-failure; however, risk stratification to identify patients at risk of progressing to end-stage HCM remains insufficient.

OBJECTIVES In this study, the authors sought to elucidate whether the coexistence of other cardiovascular disease (CVD)–related variants is associated with progression to end-stage HCM in patients with HCM harboring pathogenic or likely pathogenic (P/LP) sarcomeric variants.

METHODS The authors performed genetic analysis of 83 CVD-related genes in HCM patients from a Japanese multicenter cohort. P/LP variants in 8 major sarcomeric genes (*MYBPC3*, *MYH7*, *TNNT2*, *TNNI3*, *TPM1*, *MYL2*, *MYL3*, and *ACTC1*) definitive for HCM were defined as "sarcomeric variants." In addition, P/LP variants associated with other CVDs, such as dilated cardiomyopathy and arrhythmogenic cardiomyopathy, were referred to as "other CVD-related variants."

RESULTS Among 394 HCM patients, 139 carried P/LP sarcomeric variants: 11 (7.9%) carried other CVD-related variants, 6 (4.3%) multiple sarcomeric variants, and 122 (87.8%) single sarcomeric variants. In a multivariable Cox regression analysis, presence of multiple sarcomeric variants (adjusted HR [aHR]: 3.35 [95% CI: 1.25-8.95]; $P = 0.016$) and coexistence of other CVD-related variants (aHR: 2.80 [95% CI: 1.16-6.78]; $P = 0.022$) were independently associated with progression to end-stage HCM. Coexisting other CVD-related variants were also associated with heart failure events (aHR: 2.75 [95% CI: 1.27-5.94]; $P = 0.010$).

CONCLUSIONS Approximately 8% of sarcomeric HCM patients carried other CVD-related variants, which were associated with progression to end-stage HCM and heart failure events. Comprehensive surveillance of CVD-related variants within sarcomeric HCM patients contributes to risk stratification and understanding of mechanisms underlying end-stage HCM. (JACC Heart Fail. 2024;12:2041-2052) 8© 2024 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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ABBREVIATIONS AND ACRONYMS

ACM = arrhythmogenic cardiomyopathy

CVD = cardiovascular disease

DCM = dilated cardiomyopathy

HCM = hypertrophic cardiomyopathy

LV = left ventricle

P/LP = pathogenic or likely pathogenic

VIF = variance inflation factor

VUS = variant of uncertain significance

Hypertrophic cardiomyopathy (HCM) is a common inherited cardiovascular disease (CVD), with a prevalence of up to 1 in 500 individuals in the general population.[1,2] Patients with HCM exhibit a range of clinical manifestations, including heart failure, syncopal episodes, and lethal arrhythmias. Importantly, 4.8% to 8.1% of patients with HCM progress to end-stage HCM, characterized by systolic dysfunction frequently accompanied by left ventricular (LV) remodeling and drug-resistant heart failure.[3,4] There is a clear need to better understand end-stage HCM to improve risk stratification and clinical management.

Over the past 3 decades, molecular genetic investigations have revealed that HCM primarily arises from pathogenic variants of genes encoding sarcomeric proteins. To date, variants in 8 major sarcomeric genes have been definitively associated with HCM (*MYBPC3*, *MYH7*, *TNNT2*, *TNNI3*, *TPM1*, *MLY2*, *MYL3*, and *ACTC1*).[5,6] Pathogenic or likely pathogenic (P/LP) variants of these genes are identified in 30% to 60% of patients with HCM. Individuals harboring these sarcomeric P/LP variants demonstrate a higher incidence of cardiovascular events and progression to end-stage HCM than those without the variants.[4,7] Notably, 2.6% to 7.2% of patients with HCM harbor multiple sarcomeric P/LP variants, presenting with earlier disease onset, more severe hypertrophy, and poorer prognosis than those with single sarcomeric P/LP variants.[4,8-11]

As described earlier, HCM has been considered as a "sarcomeric disease;" however, variants in non-sarcomeric genes encoding cytoskeleton-, sarcoplasmic reticulum-, and ubiquitin ligase-related proteins also have been revealed to be associated with HCM onset.[12] Moreover, P/LP variants associated with other cardiovascular diseases (CVDs), such as dilated cardiomyopathy (DCM) and arrhythmogenic cardiomyopathy (ACM), have been identified in patients with HCM alongside sarcomeric P/LP variants.[13,14] However, these P/LP variants associated with other CVDs have never been highlighted, because they are not recognized as the primary cause of HCM and are typically not included in conventional gene panels for HCM genetic testing.[6] The role of these variants as bystanders or modifiers in patients with HCM has not yet been fully elucidated. Therefore, this study aimed to investigate the impact of the coexisting other CVD-related variants on phenotypes and clinical outcomes in patients with HCM harboring sarcomeric variants.

METHODS

STUDY POPULATION AND STUDY DESIGN. A total of 394 Japanese probands with familial or sporadic HCM in a Japanese multicenter cohort (The University of Tokyo, Kochi University, Tokyo Women's Medical University, Niigata University Graduate School of Medical and Dental Sciences, and Kyushu University) consented to genetic testing from 2003 to 2023. Clinical information was collected by physicians and assistant staff who were blinded to genetic data. Baseline characteristics, including family history, echocardiography, and cardiac magnetic resonance, represented the data from the initial evaluation at the participating institutions. A family history of HCM was determined when more than 1 family member presented with clinical evidence of HCM. A family history of sudden cardiac death included any unexpected death of a family member regardless of age. This study was approved by the Ethics Committee of the University of Tokyo Hospital (approval number G2249) and conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all of the patients.

DIAGNOSTIC CRITERIA. The diagnosis of HCM was based on 2-dimensional echocardiography findings,

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

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which included unexplained LV hypertrophy with a maximal end-diastolic LV wall thickness of ≥15 mm anywhere in the LV, or ≥13 mm in family members with a known family history of HCM.[6] Echocardiographic data were obtained by skilled and qualified sonographers, and the results were confirmed by more than 2 cardiac echocardiography experts in accordance with guideline recommendations.[6,15] Patients presenting with intra-LV gradient ≥30 mm Hg were classified as having obstructive HCM.[6,16] Patients presenting with LV hypertrophy predominantly in the apex without asymmetrical septal hypertrophy were classified as having apical HCM.[6,17] In addition, patients with LV systolic dysfunction (ejection fraction <50%) were defined as having end-stage HCM.[3,4,6]

GENETIC ANALYSIS. DNA was extracted from whole blood samples from all participants. We conducted targeted sequencing in 248 patients with the use of a custom panel targeting exons and splicing regions across 83 genes related to various CVDs.[18] The genes are listed in Supplemental Table 1 with curation from the ClinGen and ClinVar database.[19,20] The remaining 146 patients underwent whole-exome sequencing. Target genes were restricted to the same 83 genes from the gene panel used in target sequencing. Pathogenicity of the variants was evaluated and classified as pathogenic, likely pathogenic, variant of uncertain significance (VUS), benign, or likely benign variant, according to the American College of Medical Genetics and Genomics consensus guidelines.[21] Detailed methods are presented in the Supplemental Methods.

SARCOMERIC VARIANTS AND OTHER CVD-RELATED VARIANTS. P/LP variants in 8 major sarcomeric genes definitive for HCM onset were defined as "sarcomeric variants" in accordance with the definition established in previous studies.[3,4,6] These genes are myosin-binding protein C (*MYBPC3*), β-myosin heavy chain (*MYH7*), cardiac troponin T (*TNNT2*), cardiac troponin I (*TNNI3*), α-tropomyosin (*TPM1*), myosin regulatory light chain (*MLY2*), myosin essential light chain (*MYL3*), and cardiac α-actin (*ACTC1*). *MYBPC3*, *MYH7*, *MYL2*, and *MYL3* were categorized as thick filaments, whereas *TNNT2*, *TNNI3*, *TPM1*, and *ACTC1* were categorized as thin filaments. Patients with VUS and likely benign or benign variants and those without sarcomeric variants were referred to as "sarcomeric variants negative." Multiple sarcomeric variants included homozygous, compound heterozygous, and double heterozygous P/LP variants in 8 major sarcomeric genes.[22] Moreover, P/LP variants associated with other CVDs, such as DCM, ACM, LV noncompaction and arrhythmias, were referred to as "other CVD-related variants." Patients were excluded from analysis if they had P/LP variants in *LAMP2*, *GLA*, *PRKAG2*, *DES*, and *TTR*, because these indicate the presence of storage or systemic diseases that are phenocopies of HCM.

OUTCOME DEFINITIONS. The primary endpoint was defined as progression to end-stage HCM (LV ejection fraction <50%) during follow-up.[3,4,6] In addition, HCM-related composite endpoints were defined as follows: 1) an arrhythmic composite endpoint consisting of sudden cardiac death, successful resuscitation from sustained ventricular tachycardia, or fibrillation and appropriate discharge of the implantable cardioverter-defibrillator; and 2) heart failure composite endpoint consisting of hospitalization for heart failure, LV assist device implantation, heart transplantation, and heart failure-related death.

STATISTICAL ANALYSIS. Categoric variables are presented as n (%), continuous variables as median (Q1-Q3). Comparisons of continuous variables and categoric variables were performed using the Mann-Whitney *U*-test and Fisher test, respectively. For survival analysis, Kaplan-Meier curves were plotted from birth to demonstrate the cumulative incidences of study endpoints: end-stage HCM and HCM-related composite endpoints. The log-rank test was used for comparisons between groups. To identify the predictors of study endpoints, candidate variables were analyzed with the use of a Cox proportional hazard model. The proportional hazard assumption was assessed graphically and with the use of a global test based on Schoenfeld residuals ($P < 0.05$ met the assumption). Multicollinearity in the regression coefficients was assessed by means of the variance inflation factor (VIF) with a criterion of VIF <10. In the multivariable analysis, variables were incorporated into the model based on univariate analysis and clinical considerations. Predictors of study endpoints are expressed as HRs with 95% CIs. Statistical significance was defined as 2-sided $P \leq 0.05$. Multiple comparison was considered in each pairwise comparison among the 3 groups, where *P* values obtained from Mann-Whitney *U*-test and Fisher test were adjusted using the Benjamini-Hochberg procedure.[23] Sensitivity analyses were conducted to assess the robustness of our findings. Additional details of the sensitivity analyses are presented in the Supplemental Methods. All statistical analyses were conducted with JMP software version 17.0.0 (SAS Institute) and R software version 4.3.1 (R Foundation for Statistical Computing).

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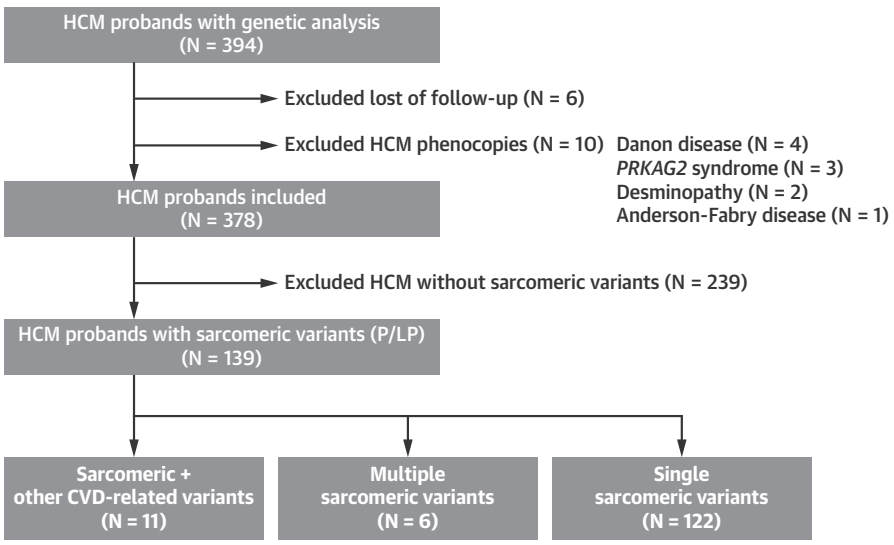
FIGURE 1 Patient Flow in the Study



CVD = cardiovascular disease; HCM = hypertrophic cardiomyopathy; LP = likely pathogenic; P = pathogenic.

RESULTS

STUDY PARTICIPANTS AND PROFILE OF OTHER CVD-RELATED GENES. Among the 394 patients with HCM, 6 who were lost to follow-up were excluded. Ten patients with HCM phenocopies also were excluded: 4 with Danon disease, 3 with *PRKAG2* syndrome, 2 with desminopathy, and 1 with Anderson-Fabry disease (**Figure 1**). Of the remaining 378 patients, 139 (36.8%) presented with 76 sarcomeric P/LP variants: 23 with *MYBPC3*, 33 with *MYH7*, 3 with *TNNT2*, 6 with *TNNI3*, 3 with *TPM1*, 2 with *MYL2*, 2 with *MYL3*, and 4 with *ACTC1*. All P/LP variants and VUS in sarcomeric genes are listed in Supplemental Table 2. Patients with sarcomeric variants exhibited a higher incidence of progression to end-stage HCM and HCM-related composite endpoints compared with those without sarcomeric variants (Supplemental Figure 1). Among the 139 patients with sarcomeric variants, 11 (7.9%) also carried other CVD-related variants alongside single sarcomeric variants, 6 (4.3%) harbored multiple sarcomeric variants, and 122 (87.8%) had single sarcomeric variants (**Central Illustration**).

The variants identified in the 11 patients with both sarcomeric and other CVD-related variants are listed in **Table 1**. The sarcomeric variants included 10 variants: 3 missense variants in *MYH7*, 1 nonsense variant in *MYBPC3*, 1 frameshift deletion variant in *MYBPC3*, 1 canonical splice-site variant (donor loss) in *MYBPC3*, and missense variants in *TNNT2*, *TNNI3*, *MYL3*, and *ACTC1*. The other CVD-related variants included 10 variants: 1 frameshift deletion variant in *TTN*, 1 missense variant in *FLNC*, 1 in-frame deletion variant in *BAG3*, 1 frameshift insertion variant and 1 nonsense variant in *NEBL*, 1 frameshift deletion variant in *NKX2-5*, 1 canonical splice-site variant (acceptor loss) in *MIB1*, 1 nonsense variant in *ABCC9*, 1 frameshift deletion variant in *TGFB3*, and 1 frameshift deletion variant in *KCNA5*. All variants were double heterozygous. Interpretation and classification of the pathogenicity of the other CVD-related variants are presented in Supplemental Table 3.

Among the 6 patients with multiple sarcomeric variants, 4 had homozygous variants: 3 missense variants in *MYBPC3* and 1 missense variant in *MYH7* (**Table 2**). One patient had compound heterozygous variants within *MYBPC3* (a missense variant and a splice-site variant), and 1 had double heterozygous missense variants within *MYBPC3* and *MYH7*.

BASELINE CHARACTERISTICS. Patient characteristics at the initial evaluation are listed in **Table 3**.

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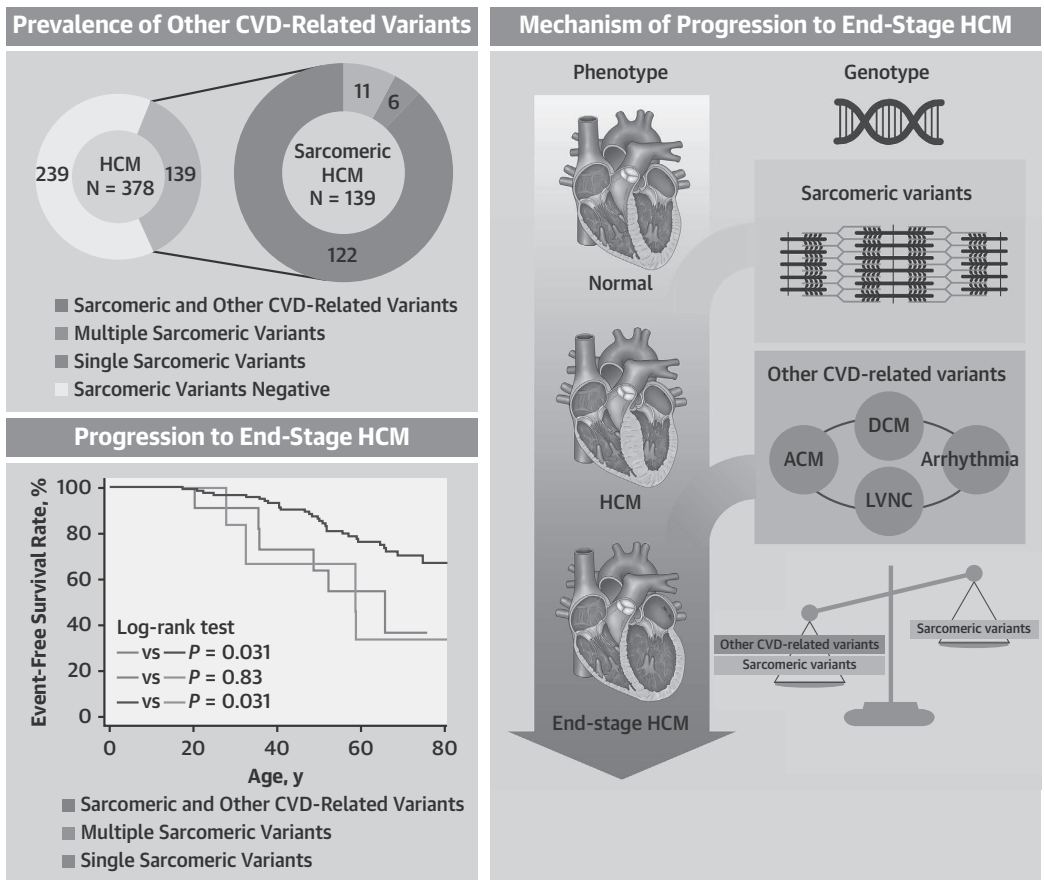
CENTRAL ILLUSTRATION Significance of Multiple Variants in HCM



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(Top left) Among 378 patients with HCM from a Japanese multicenter cohort, 139 patients harboring sarcomeric variants were included. Of these, 11 patients harbored other cardiovascular disease (CVD)-related pathogenic or likely pathogenic variants. (Bottom left) Patients harboring both sarcomeric and other CVD-related variants exhibited a higher rate of progression to end-stage HCM than patients with single sarcomeric variants. (Right) Sarcomeric variants are the primary cause of HCM onset, and other CVD-related variants act as modifiers of phenotypes and clinical outcomes in patients with sarcomeric variants. ACM = arrhythmogenic cardiomyopathy; DCM = dilated cardiomyopathy; HCM = hypertrophic cardiomyopathy; LVNC = left ventricular noncompaction.

Patients with the coexistence of other CVD-related variants were diagnosed with HCM at a median age of 34 years (Q1-Q3: 20-50 years) and underwent initial evaluation at the participating institutions at 50 years (Q1-Q3: 40-52 years) of age. Five (45.5%) were male, and 5 (45.5%) had a family history of HCM. Eight patients (72.7%) had nonobstructive HCM, and 3 (27.3%) had end-stage HCM. Implantable cardioverter-defibrillators and cardiac resynchronization therapy defibrillators were each implanted in 2 patients (18.2%). None of the patients underwent septal reduction therapy. Regarding the patients' characteristics, no significant differences were observed in patients with the coexistence of other CVD-related variants compared with either those with single or those with multiple sarcomeric variants. Further

TABLE 1 List of the Identified Variants From 11 Patients With Sarcomeric and Other CVD-Related Variants

	First Variant in Sarcomeric Genes					Second Variant in Other CVD-Related Genes				
Case	Gene	Coding DNA	Protein	Type	ACMG	Gene	Coding DNA	Protein	Type	ACMG
PT_01	*MYH7*	c.G2536A	p.E846K	Missense	LP	*TTN*	c.13934_13937del	p.D4645Vfs*24	Frameshift	LP
PT_02	*TNNT2*	c.T328A	p.F110I	Missense	P	*FLNC*	c.G6958A	p.G2320R	Missense	LP
PT_03	*MYBPC3*	c.C890G	p.S297*	Nonsense	P	*BAG3*	c.780_794del	p.R261_P265del	In-frame	LP
PT_04	*MYBPC3*	c.1777delT	p.S593Pfs*9	Frameshift	P	*NEBL*	c.2120dupC	p.E709Rfs*26	Frameshift	LP
PT_05	*ACTC1*	c.G301A	p.E101K	Missense	P	*NEBL*	c.2120dupC	p.E709Rfs*26	Frameshift	LP
PT_06	*MYL3*	c.G281A	p.R94H	Missense	LP	*NEBL*	c.C2632T	p.R878*	Nonsense	LP
PT_07	*MYBPC3*	c.1790+1G >T	—	Splicing	P	*NKX2-5*	c.A386del	p.A128_L129ins*	Frameshift	LP
PT_08	*MYBPC3*	c.1777delT	p.S593Pfs*9	Frameshift	P	*MIB1*	c.2050-1G >A	—	Splicing	LP
PT_09	*MYH7*	c.C5279G	p.T1760R	Missense	LP	*ABCC9*	c.4571delT	p.L1524*	Nonsense	LP
PT_10	*MYH7*	c.G746A	p.R249Q	Missense	P	*TGFB3*	c.572delT	p.L191Rfs*23	Frameshift	LP
PT_11	*TNNI3*	c.G557A	p.R186Q	Missense	P	*KCNA5*	c.1103_1110del	p.F369Pfs*87	Frameshift	LP

ACMG = American College of Medical Genetics and Genomics; CVD = cardiovascular disease; LP = likely pathogenic; P = pathogenic.

detailed data of patients with the coexistence of other CVD-related variants and multiple sarcomeric variants are presented in Supplemental Tables 4 and 5, respectively.

PREDICTIVE FACTORS FOR PROGRESSION TO END-STAGE HCM. At the last evaluation, a total of 40 patients (28.8%) had progressed to end-stage HCM: 6 (54.5%) of the 11 patients with coexistence of other CVD-related variants, 5 (83.3%) of the 6 patients with multiple sarcomeric variants, and 29 (23.8%) of the 122 patients with single sarcomeric variants. The duration from HCM diagnosis to progression to end-stage HCM was 6.5 years (Q1-Q3: 0.0-18.1 years). The prevalence of progression to end-stage HCM in patients with the coexistence of other CVD-related variants was notably higher than in patients with single sarcomeric variants, according to Kaplan-Meier analysis from birth to the last evaluation (log-rank $P = 0.031$), whereas it was similar to that in patients with multiple sarcomeric variants (log-rank $P = 0.83$) (**Figure 2**). After univariable analysis, male sex, presence of multiple sarcomeric variants, and the coexistence of other CVD-related variants were included in the multivariate Cox regression analysis (**Table 4**). Schoenfeld residual plots are presented in Supplemental Figure 2. The mean VIF was 1.02. Consequently, both the presence of multiple sarcomeric variants (adjusted HR: 3.35 [95% CI: 1.25-8.95]; $P = 0.016$) and the coexistence of other CVD-related variants (adjusted HR: 2.80 [95% CI: 1.16-6.78]; $P = 0.022$) were independently associated with progression to end-stage HCM among patients with sarcomeric variants.

ASSOCIATION WITH HCM-RELATED COMPOSITE ENDPOINTS. At the last evaluation, 29 arrhythmic and 61 heart failure composite endpoints were observed. Among patients with the coexistence of other CVD-related variants, 1 had sustained ventricular tachycardia, 8 were hospitalized for heart failure, and 2 had LV assist device implantation and heart transplantation. The incidence of the arrhythmic composite endpoint was similar among the 3 groups, whereas the incidence of the heart failure composite endpoint was higher in patients with the coexistence of other CVD-related variants than in those with single sarcomeric variants (log-rank $P = 0.014$) (**Figure 3**). After multivariable Cox regression analysis, the coexistence of

TABLE 2 List of the Identified Variants From 6 Patients With Multiple Sarcomeric Variants

	First Variant in Sarcomeric Genes					Second Variant in Sarcomeric Genes				
Case	Gene	Coding DNA	Protein	Type	ACMG	Gene	Coding DNA	Protein	Type	ACMG
PT_A	*MYBPC3*	c.C1112T	p.P371L	Missense	LP	*MYBPC3*	c.C1112T	p.P371L	Missense	LP
PT_B	*MYBPC3*	c.T2285A	p.V762D	Missense	LP	*MYBPC3*	c.T2285A	p.V762D	Missense	LP
PT_C	*MYBPC3*	c.G2459A	p.R820Q	Missense	LP	*MYBPC3*	c.G2459A	p.R820Q	Missense	LP
PT_D	*MYH7*	c.C2608T	p.R870C	Missense	LP	*MYH7*	c.C2608T	p.R870C	Missense	LP
PT_E	*MYBPC3*	c.G2459A	p.R820Q	Missense	LP	*MYBPC3*	c.2905+1G >A	—	Splicing	P
PT_F	*MYBPC3*	c.G2459A	p.R820Q	Missense	LP	*MYH7*	c.C1686A	p.N562K	Missense	LP

Abbreviations as in Table 1.

TABLE 3 Patient Characteristics at Initial Evaluation

	Sarcomeric and Other CVD-Related Variants (n = 11)	MULTIPLE Sarcomeric Variants (n = 6)	SINGLE Sarcomeric Variants (n = 122)	*P* Value[a]	*P* Value[b]	*P* Value[c]
Age at diagnosis of HCM, y	34 (20-50)	45 (20-69)	45 (32-59)	0.69	0.27	0.99
Age at initial evaluation, y	50 (40-52)	58 (47-76)	56 (39-66)	0.36	0.36	0.45
Male	5 (45.5)	2 (33.3)	67 (54.9)	1.00	1.00	1.00
Family history of HCM	5 (45.5)	4 (66.7)	58 (47.5)	1.00	1.00	1.00
Family history of SCD	0 (0.0)	1 (16.7)	22 (18.0)	0.71	0.63	1.00
Phenotype				1.00	1.00	1.00
Nonobstructive HCM, %	8 (72.7)	2 (33.3)	72 (59.0)			
Obstructive HCM, %	0 (0.0)	0 (0.0)	20 (16.4)			
Apical HCM, %	0 (0.0)	0 (0.0)	5 (4.1)			
End-stage HCM, %	3 (27.3)	4 (66.7)	25 (20.5)			
NYHA functional class III/IV	2 (18.2)	4 (66.7)	22 (18.0)	0.22	1.00	0.046
Unexpected syncope	3 (27.3)	1 (16.7)	21 (17.2)	1.00	1.00	1.00
Atrial fibrillation	6 (54.5)	5 (83.3)	40 (32.8)	0.33	0.33	0.061
NSVT	8 (72.7)	3 (50.0)	40 (32.8)	0.60	0.052	0.60
Echocardiographic parameters						
Interventricular septal wall thickness, mm	13 (10-22)	13 (12-17)	16 (12-19)	1.00	0.57	0.57
LV posterior wall thickness, mm	11 (9-13)	11 (8-12)	10 (9-12)	0.81	0.57	0.81
LV end-diastolic diameter, mm	43 (40-56)	49 (42-56)	45 (40-50)	0.67	0.83	0.67
LV end-systolic diameter, mm	28 (19-38)	35 (23-43)	28 (22-34)	0.76	0.76	0.76
LV ejection fraction, %	60 (42-66)	42 (36-65)	64 (54-72)	0.37	0.34	0.22
Left atrial diameter, mm	48 (42-50)	45 (34-52)	44 (38-50)	0.92	0.92	0.92
Maximum LV wall thickness, mm	14 (12-24)	15 (14-21)	18 (15-21)	0.72	0.72	0.72
Device therapy						
Pacemaker implantation	0 (0.0)	0 (0.0)	3 (2.5)	1.00	1.00	1.00
ICD implantation	2 (18.2)	2 (33.3)	13 (10.7)	0.58	0.58	0.44
CRT-D implantation	2 (18.2)	1 (16.7)	4 (3.3)	1.00	0.23	0.43
Septal reduction therapy						
Myectomy	0 (0.0)	0 (0.0)	2 (1.6)	1.00	1.00	1.00
Alcohol septal ablation	0 (0.0)	0 (0.0)	0 (0.0)	1.00	1.00	1.00

Values are median (Q1-Q3) or n (%). *P* values obtained from Mann-Whitney *U*-test and Fisher test were adjusted with the Benjamini-Hochberg procedure. [a]Comparison between sarcomeric and other CVD-related variants vs multiple sarcomeric variants. [b]Comparison between sarcomeric and other CVD-related variants vs single sarcomeric variants. [c]Comparison between single sarcomeric variants vs multiple sarcomeric variants.

CRT-D = cardiac resynchronization therapy defibrillator; HCM = hypertrophic cardiomyopathy; ICD = implantable cardioverter-defibrillator; LV = left ventricular; NSVT = nonsustained ventricular tachycardia; SCD = sudden cardiac death; other abbreviation as in Table 1.

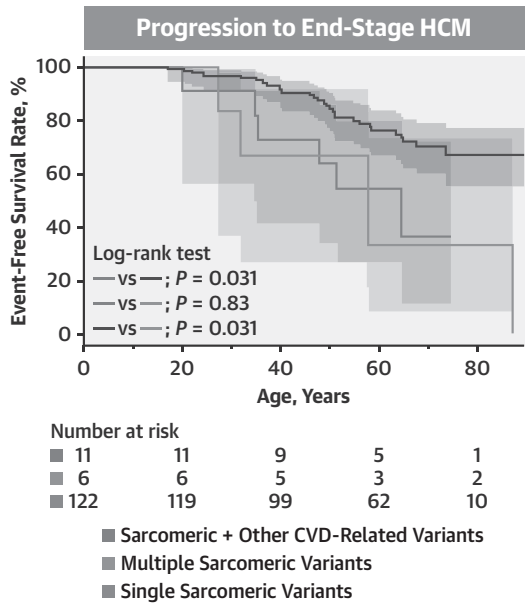
other CVD-related variants (adjusted HR: 2.75 [95% CI: 1.27-5.94]; $P = 0.010$) was independently associated with the heart failure composite endpoint (**Table 5**). Schoenfeld residual plots are presented in Supplemental Figure 3. The mean VIF was 1.10.

SENSITIVITY ANALYSES. Sensitivity analyses expanding the definition of "sarcomeric variants" including VUS in addition to P/LP (sensitivity analysis 1, Supplemental Figure 5) and "other CVD-related variants" including both predicted loss-of-function and putative pathogenic missense variants (sensitivity analysis 2, Supplemental Figure 10) revealed that the coexistence of other CVD-related variants was independently associated with progression to end-stage HCM and the heart failure composite endpoint in patients with sarcomeric HCM (Supplemental Tables 6 to 16, Supplemental Figures 6 to 9, 11 to 13). Moreover, the patients with multiple variants in different genes exhibited higher incidence of progression to end-stage HCM and HCM-related composite endpoints compared with those with single/multiple variants in the same genes (sensitivity analysis 3, Supplemental Tables 17 to 20, Supplemental Figures 14 to 18). Additional details of the sensitivity analyses are presented in the Supplemental Methods.

DISCUSSION

In this study, we conducted genetic analysis targeting 83 CVD-related genes in 394 patients with HCM from a Japanese multicenter cohort. Of these, we focused on 139 patients who carried major sarcomeric variants definitive for HCM onset. The main findings of this study are that: 1) approximately 8% of patients with

FIGURE 2 Kaplan-Meier Analysis for Progression to End-Stage HCM



Patients with other cardiovascular disease–related variants exhibited a higher incidence of progression to end-stage HCM than those with single sarcomeric variants. Abbreviation as in Figure 1.

HCM harboring sarcomeric variants also harbored other CVD-related variants; and 2) the presence of these other CVD-related variants was an independent risk factor for progression to end-stage HCM and heart failure events. The robustness of our findings was underscored by additional sensitivity analyses.

RELATIONSHIP BETWEEN SARCOMERIC VARIANTS AND END-STAGE HCM. Previous research has extensively examined the variants of 8 major sarcomeric genes. The presence of sarcomeric variants, particularly those in thin filaments, has been identified as a risk factor for progression to end-stage HCM.[4] Concerning thick filaments, *MYBPC3*-truncating variants, primarily associated with haplotype insufficiency, showed a higher propensity for end-stage HCM compared with variants in *MYH7*, which predominantly exhibited a dominant-negative effect.[24,25] Furthermore, patients with HCM harboring multiple sarcomeric variants exhibited earlier disease onset, more severe hypertrophy, and a higher incidence of cardiovascular events and progression to end-stage HCM.[8-11] These pronounced morphologic changes and adverse prognoses were thought to result from the cumulative effects of multiple sarcomeric variants,[11] in agreement with our HCM cohort, in which a higher incidence of progression to end-stage HCM and the heart failure composite endpoint were observed. Nevertheless, the impact of other CVD-related variants has not been systematically investigated in patients with sarcomeric HCM, because they are not generally recognized as the primary cause of disease onset.

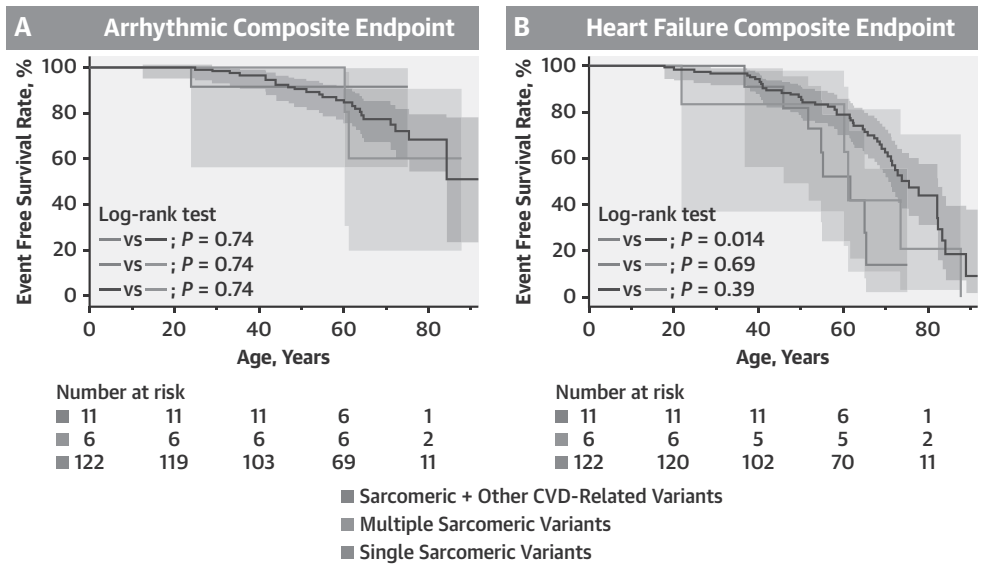
ROLES OF OTHER CVD-RELATED VARIANTS. In this study, we demonstrated the distribution and prognostic impact of variants in genes related to various CVDs, which may have been underestimated by conventional analyses using gene panels focusing on sarcomeric genes. Indeed, we identified approximately 8% of patients with sarcomeric HCM also harboring other CVD-related variants, which was more prevalent than multiple sarcomeric variants (4.3%) in our HCM cohort. The CVD-related variants

TABLE 4 Predictors of the Progression to End-Stage HCM in Univariate and Multivariable Cox Regression Analyses

	Univariate			Multivariable		
	HR	95% CI	*P* Value	Adjusted HR	95% CI	*P* Value
Male	1.53	0.81-2.89	0.19	1.54	0.81-2.90	0.18
Family history of HCM	1.36	0.73-2.54	0.34			
Family history of SCD	0.99	0.41-2.36	0.98			
Thick-filament gene variants	0.63	0.28-1.43	0.27			
MYBPC3 variants	0.98	0.53-1.83	0.95			
MYH7 variants	0.81	0.42-1.57	0.53			
Thin-filament gene variants	1.58	0.70-3.59	0.27			
Variants classification						
Single sarcomeric variants (Ref.)	1.00	–	–	1.00	–	–
Multiple sarcomeric variants	3.38	1.14-6.66	0.015	3.35	1.25-8.95	0.016
Other CVD-related variants	2.76	1.34-6.66	0.024	2.80	1.16-6.78	0.022

Ref. = Reference; other abbreviations as in Tables 1 and 3.

FIGURE 3 Kaplan-Meier Analysis for Arrhythmic and Heart Failure Composite Endpoints



The incidence of the arrhythmic composite endpoint was similar among the 3 groups (A), whereas the incidence of the heart failure composite endpoint was higher in patients with the coexistence of other cardiovascular disease (CVD)-related variants than in those with single sarcomeric variants (B). Median survival time of the heart failure composite endpoint in patients with single sarcomeric variants was 75.3 years (95% CI: 71.1-83.2 years).

identified in this study exhibited diversity, encoding sarcomere-, cytoskeleton-, co-chaperone-, ion channel-, transcription factor-, and signaling pathway-related proteins. The other CVD-related variants were categorized into 3 groups: variants in major cardiomyopathy-related genes (*TTN*, *FLNC*, and *BAG3*), minor cardiomyopathy-related genes (*ABCC9*, *MIB1*, *NEBL*, *NKX2-5*, and *TGFB3*), and arrhythmia-related genes (*KCNA5*). At the last evaluation, patients with variants in *TTN*, *FLNC*, *NEBL*, *TGFB3*, and *KCNA5* had progressed to end-stage HCM.

TABLE 5 Predictors of the Heart Failure Composite Endpoint in Univariate and Multivariable Cox Regression Analyses

	Univariate			Multivariable		
	HR	95% CI	*P* Value	Adjusted HR	95% CI	*P* Value
Male	1.38	0.83-2.30	0.21	1.30	0.77-2.21	0.33
Family history of HCM	1.36	0.82-2.27	0.23			
Family history of SCD	0.90	0.41-2.00	0.80			
Thick-filament gene variants	0.60	0.30-1.19	0.15			
MYBPC3 variants	0.59	0.35-0.99	0.049	0.63	0.36-1.12	0.12
MYH7 variants	1.37	0.82-2.28	0.23			
Thin-filament gene variants	1.67	0.84-3.32	0.15	1.21	0.56-2.57	0.63
Variants classification						
Single sarcomeric variants (Ref.)	1.00	–	–	1.00	–	–
Multiple sarcomeric variants	1.60	0.63-4.10	0.36	1.56	0.57-4.25	0.38
Other CVD-related variants	2.75	1.28-5.98	0.010	2.75	1.27-5.94	0.010

Abbreviations as in Tables 1, 3, and 4.

Major cardiomyopathy-related genes have definitive/strong evidence for cardiomyopathies (Supplemental Table 1). *TTN* and *BAG3* are well established DCM-related genes that encode titin and antiapoptotic co-chaperone protein, respectively.[26,27] *FLNC* encodes an actin-binding protein and serves as a definitive gene for DCM. Variants in *FLNC*, on the other hand, exhibit phenotypic diversity. Missense variants in *FLNC* have been reported as P/LP for DCM, HCM, and restrictive cardiomyopathy. In addition, truncating variants in *FLNC* have been reported as P/LP for DCM, HCM, restrictive cardiomyopathy, and ACM.[28,29] Variants in these major cardiomyopathy-related genes might affect phenotypes even in patients with HCM.

Minor cardiomyopathy-related genes were reported to be associated with various cardiomyopathies, whereas their evidence is currently limited to small-scale studies or animal models (Supplemental Table 1). Truncating variants in these genes are expected to induce null effect and act as potential predisposition to cardiomyopathy.[30-38] In the present study, 2 of the 3 patients with a sarcomeric variant and a predicted loss-of-function variant in *NEBL* developed end-stage HCM. *NEBL* encodes the nebulette protein, predominantly expressed in cardiomyocytes, serving a pivotal role in sarcomere organization.[30] Variants in *NEBL* result in disorganization of sarcomere and are associated with various forms of cardiomyopathies.[31,32] Knockout mouse models of *NEBL* demonstrated reduced cardiac tolerance to biomechanical stress and complicated adverse LV remodeling.[33] Given the essential role of nebulette in myofibril function, truncating variants in *NEBL* are expected to have significant consequences. Indeed, a frameshift deletion variant in *NEBL* is classified as likely pathogenic for DCM in the ClinVar database.[20] The patient with *MYH7* missense and *TGFB3* frameshift deletion variants had also progressed to end-stage HCM. *TGFB3* encodes the secreted ligand of the transforming growth factor-β superfamily proteins, and its truncating variants are well known causes of Loeys-Dietz syndrome; they are also associated with ACM.[34,35] Arrhythmia-related genes included *KCNA5*, which has been reported to be associated with atrial fibrillation (Supplemental Table 1). *KCNA5* encodes a potassium voltage-gated channel, and its truncating variants cause repolarization deficiency in atrial myocytes, leading to atrial fibrillation.[39] Although the relationship between *KCNA5* and cardiomyopathies remains elusive, the presence of atrial fibrillation itself deteriorates LV systolic function.[3]

INVESTIGATING OTHER CVD-RELATED VARIANTS REFINES RISK STRATIFICATION. In the whole-exome (or -genome) sequencing for HCM patients, the rare variants that are not definitive for HCM onset but are associated with various other cardiovascular phenotypes are frequently identified alongside sarcomeric variants.[14] Although the molecular biological effect of nonsarcomeric variants in patients with sarcomeric variants has been unclarified, the present study reveals a novel insight. We found that the coexistence of variants associated with various cardiomyopathies or arrhythmias could potentially modify phenotypes and clinical outcomes in individuals with sarcomeric variants (**Central Illustration**). Comprehensive surveillance of HCM and other CVD-related variants contribute to refining risk stratification, especially identification of patients who are at high risk for progression to end-stage HCM and heart failure events. Therefore, the role of the "second variants" alongside sarcomeric variants should not be overlooked, because they have more than just a bystanders' effect and warrant thorough investigation and evaluation.

Although the coexistence of other CVD-related variants was associated with end-stage HCM, the underlying mechanisms of progression to end-stage HCM remains elusive. One of the distinguishing features of end-stage HCM is extensive myocardial fibrotic changes.[40] Transgenic mouse models harboring multiple sarcomeric variants exhibited severe hypertrophy with extensive myocardial fibrosis.[41] These findings have been thought to be caused by cardiac ischemia due to microvasculopathy, an intrinsic feature of HCM.[42] Extensive myocardial fibrosis was also observed in patients with the coexistence of other CVD-related variants, as shown in the representative cardiac magnetic resonance in Supplemental Figure 4. Although the pathway leading to myocardial fibrosis remains unclear in this population, myocardial fibrosis would play a pivotal role in progression to end-stage HCM in patients with the coexistence of other CVD-related variants. Looking to the future, functional analyses using different modalities, including nonhuman animal models, should be conducted to elucidate the interaction between sarcomeric variants and other CVD-related variants.

STUDY LIMITATIONS. We acknowledge that the present study has certain methodologic limitations that should be considered. The primary limitation is a small subcohort size of the multiple variants and the lack of a validation study. Although the main results were consistent through our sensitivity analyses, a larger-scale study with a replication cohort is needed

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to confirm the robustness of our findings. In addition, expanding the gene panel to include more genes may lead to the identification of a greater number of CVD-related variants. Second, evidence of the minor cardiomyopathy-related variants as potential predispositions for cardiomyopathy is limited to small studies or nonhuman animal models. Advances in gene analyses from Sanger sequencing to next-generation sequencing have contributed to the identification of variants in such unexpected "minor cardiomyopathy-related genes." Indeed, the presence of variants in minor cardiomyopathy-related genes has become more apparent in the whole-exome sequencing era; however, functional analyses for these genes remain to be conducted. Third, survivorship bias could have influenced the results of this study because patients must survive until they are seen at the participating institutions. Fourth, referral bias also could have affected our study, because the participating institutions included advanced medical centers where heart transplantation procedures were conducted. Consequently, the prevalence of end-stage HCM in our HCM cohort was higher than in previous reports.[3,4]

CONCLUSIONS

HCM primarily arises from major sarcomeric variants; however, it is noteworthy that approximately 8% of patients with sarcomeric HCM have both sarcomeric and other CVD-related variants. These additional variants were reported to be associated with a wide range of cardiovascular phenotypes, including DCM, ACM, LV noncompaction, and arrhythmias. The present study suggests that the coexistence of other CVD-related variants increases the likelihood of progression to end-stage HCM and occurrence of heart failure-related events. These findings underscore the importance of conducting comprehensive surveillance of CVD-related variants to elucidate the diversity of genetic causes of HCM and facilitate precision care strategies.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE 1: Approximately 8% of patients with HCM harboring P/LP variants in major sarcomere-encoding genes definitive for HCM also carry P/LP variants associated with other CVDs, such as dilated cardiomyopathy and arrhythmogenic cardiomyopathy.

COMPETENCY IN MEDICAL KNOWLEDGE 2: Coexisting additional CVD-related variants are not just bystanders but independent prognostic factors for progression to end-stage HCM and heart failure events in patients with sarcomeric variants. Conducting comprehensive surveillance of CVD-related variants contributes to refining risk stratification and precision medicine, even in patients with sarcomeric HCM.

TRANSLATIONAL OUTLOOK: A large-scale cohort study with replication cohort is needed to confirm our findings. Moreover, further investigation using nonhuman animal models, human-induced pluripotent stem myocardial cells, and human myocardial tissues should be conducted to better understand the underlying mechanisms of progression to end-stage HCM.

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APPENDIX For an expanded Methods section as well as supplemental figures, tables, and references, please see the online version of this paper.