Late Gadolinium Enhancement for Prediction of Mutation-Positive Hypertrophic Cardiomyopathy on the Basis of Panel-Wide Sequencing

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Background: Cardiac magnetic resonance (CMR) with late gadolinium enhancement (LGE) revealed a substantial variation in the extent of myocardial scarring, a pathological hallmark of hypertrophic cardiomyopathy (HCM). However, few data exist regarding the relationship between the presence of gene mutations and the extent of LGE. Therefore, we aimed to investigate whether variations in the extent of LGE in HCM patients can be explained by the presence or absence of disease-causing mutations.

Methods and Results: We analyzed data from 82 unrelated HCM patients who underwent both LGE-CMR and next-generation sequencing. We identified disease-causing sarcomere gene mutations in 44 cases (54%). The extent of LGE on CMR was an independent factor for predicting mutation-positive HCM (odds ratio 2.12 [95% confidence interval 1.51–3.83], $P < 0.01$). The area under the curve of %LGE was greater than that of the conventional Toronto score for predicting the presence of a mutation (0.96 vs. 0.69, $P < 0.01$). Sensitivity, specificity, positive predictive value, and negative predictive value of %LGE (cutoff $> 8.1\%$) were 93.2%, 89.5%, 91.1%, and 91.9%, respectively.

Conclusions: The results demonstrated that %LGE clearly discriminated mutation-positive from mutation-negative HCM in a clinically affected HCM population. HCM with few or no myocardial scars may be genetically different from HCM with a higher incidence of myocardial scars.

Key Words: Cardiac magnetic resonance imaging; Fibrosis; Hypertrophic cardiomyopathy

Hypertrophic cardiomyopathy (HCM) is characterized by unexplained left ventricular hypertrophy (LVH) caused by mutations in genes that encode sarcomere protein.¹ Myocardial fibrosis is a major histopathologic feature of HCM, which can be classified into either myocardial scar or interstitial fibrosis.^{2,3} Cardiac magnetic resonance imaging (CMR) with late gadolinium enhancement (LGE) imaging has been established as the modality for assessing myocardial scarring in HCM patients. Previous data demonstrated a substantial variation in the extent of myocardial scarring determined by LGE-CMR.^{4,5} Although it has been postulated that myocardial scar may be subsequent to cardiomyocyte death caused by myocardial ischemia and metabolic abnormalities,^{1,6} other data have indicated that myocardial scarring could be a primary expression of HCM.⁷

The development of genetic testing has enabled the identification of subgroups of HCM based on clinical and genetic information.⁸ Recent data revealed that 40% of

HCM probands did not have a family history or sarcomere gene mutations, and were classified into a new subgroup, nonfamilial HCM.⁸ Although Ellims et al⁹ showed that the presence of LGE is higher in genotype-positive HCM patients than in patients with genotype-negative HCM, it is unclear whether variations in the extent of LGE in HCM patients can be explained by the presence or absence of disease-causing mutations. Therefore, in this study, we aimed to investigate whether HCM patients with absence or a small extent of LGE constitute a previously unrecognized subgroup of HCM, by using comprehensive genetic testing of all known HCM-related genes by next-generation sequencing (NGS).

Methods

Study Population and Clinical Evaluation

Clinically affected HCM patients at Kanazawa University Hospital or its affiliate hospitals that registered in our

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Figure 1. Evaluation of the extent of late gadolinium enhancement (LGE) in delayed gadolinium-enhanced cardiac magnetic resonance (CMR) and correlations between the extent of LGE and the mutation-positive or -negative status in representative HCM patients. **(A)** Area of LGE was measured in at least 4 short-axis slices. **(B)** Basal slice of CMR demonstrated LGE on anterior–anteroseptal segments of the left ventricle. **(C)** Areas of LGE (red) at 6 SD above the normal myocardial signal were measured by ImageJ software and presented as the percentage of the LV area. **(D)** A 54-year-old male with HNCM, and a family history of sudden cardiac death, showed 13.1% LGE on CMR. Panel sequencing detected a large deletion in the MYBPC3 gene. **(E)** A 64-year-old male with HNCM showed neither LGE in his cardiac muscle nor any candidate variants. HNCM, hypertrophic non-obstructive cardiomyopathy.

cohort between 2008 and 2016 were included in the present study if they underwent CMR within 1 year of baseline echocardiography. Patients who exhibited a dilated phase at CMR evaluation were excluded. The diagnosis of HCM was based on the echocardiographic demonstration of LVH (maximal LV wall thickness ≥ 15 mm) in the absence of other cardiac or systemic causes of LVH.^{5,10} The clinical assessment included medical history, detailed physical examination, blood tests, electrocardiography, echocardiography, and CMR.

CMR

CMR consisting of standard clinical scans was performed using 1.5-Tesla magnets. The LGE images were acquired 10 min after intravenous administration of 0.2 mmol/kg of gadolinium diethylenetriaminepentaacetic acid as previously described.¹¹ Areas of LGE at 6 standard deviations (SD) above the normal myocardial signal were evaluated according to a method verified for detecting fibrosis.^{12,13} The LGE areas were measured by semiautomatic computer-assisted planimetry in each short-axis image using ImageJ by operators who were blind to genotype (**Figure 1A–C**). The %LGE was calculated by dividing the sum of the LGE areas by that of the total LV muscle areas to give the percentage. We defined LGE-positive as any level of %LGE. In the absence of established methodology, an assessment of the variability between observations and observers was conducted to validate the accuracy of the obtained %LGE value. The CMR sequences of all patients were analyzed by two trained analysts. The 1st observer

reviewed all the sequences after a 3-month interval to assess the intraobserver variability. The number of LGE segments was also evaluated using 17 myocardial segments.¹⁴

Library Preparation and Targeted Sequencing

DNA samples were isolated from peripheral white blood cells of each patient using a standard DNA extraction protocol. We designed an original gene panel that covered known HCM-related genes and genes possibly related with metabolic cascades (**Table S1**), and applied the panel to the first 15 patients. Multiplex polymerase chain reaction of the panel was performed using the Ion AmpliSeq Library Kit 2.0 (Thermo Fisher Scientific, Waltham, MA, USA). Sequencing was carried out on Ion PGM systems (Thermo Fisher Scientific). For the remaining 67 patients, we used a TruSight Cardio sequencing panel (Illumina, San Diego, CA, USA) following the manufacturer's instructions. This panel contains 174 genes that are related to cardiovascular disease, including known genes associated with familial HCM (**Table S1**). Variants, including single nucleotide polymorphisms, and short indels, were identified using the Genome Analysis Toolkit (GATK, Broad Institute, Boston, MA, USA) by calling raw variants.

Bioinformatics Filtering Methods

To identify causative variants among the HCM patients, we applied independent basic variant filtering processes that have commonly been reported in previous NGS studies.^{15,16} Variants were excluded if they did not meet the following criteria: (1) high quality based on the cutoff in

depth of coverage, strand bias, and Genotype Quality score; (2) missense or protein-truncation (nonsense, essential splice-site, or frameshift) predicted by SnpEff; and (3) minor allele frequency <0.01% referring to the Asian cohort in the 1000 Genome Project.

We defined a potentially causative mutation as pathogenic variants confirmed as follows: (1) literature review using various sources cited in the Human Mutation Database (HGMD) and ClinVar or (2) ≥ 1 of the in silico prediction tools, including SIFT (scaled score <0.05=deleterious), Polyphen2 (scaled score >20=probably damaging), and CADD (scaled C-score >20=potentially pathogenic), which integrates diverse genome annotations and predicts the pathogenicity of non-synonymous variants in silico.¹⁷ The pathogenic mutations were confirmed using the Sanger method following a standard protocol (BigDye[®] Terminator v3.1 Cycle Sequencing Kit, Thermo Fisher Scientific) as previously described.¹⁸

Statistical Analysis

All statistical analyses were conducted using SPSS Statistics (version 19, SPSS Inc., Chicago, IL, USA). Categorical variables are expressed as percentages. Continuous variables with a normal distribution are shown as the mean $\pm$ SD. To assess the intra- and interobserver agreements, Lin's concordance correlation was used in accordance with a proposal that suggests that almost perfect agreement is indicated by >0.99, whereas poor agreement is indicated by <0.9.¹⁹ Multiple logistic regression analysis was used to determine the independent predictors for mutation-positive HCM, based on Akaike's Information Criteria as model-selection criterion. Receiver-operating characteristic (ROC) analyses were used to estimate the discriminating ability for mutation-positive HCM for %LGE and the Toronto genetic risk score, which consists of age, sex, hypertension, LV morphology, and the maximal wall thickness (MWT)/posterior wall thickness ratio.⁸ Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were defined as previously reported.²⁰ A P-value <0.05 was considered statistically significant.

Table 1. Clinical Characteristics, Electrocardiographic and Echocardiographic Findings, and LGE on CMR Imaging of the 82 Study Patients With HCM

	n=82
Clinical	
Age (years)	55.4 $\pm$ 16.5
Male sex, n (%)	47 (57.3)
Familial, n (%)	29 (35.4)
Hypertension, n (%)	22 (26.8)
AF, n (%)	8 (9.8)
BNP (pg/mL)	312.2 $\pm$ 365.2
Electrocardiography	
Pathological Q wave, n (%)	4 (4.9)
Fragmented QRS, n (%)	38 (46.3)
Echocardiography	
HOcm, n (%)	16 (19.5)
Apical hypertrophy, n (%)	12 (14.6)
LAD (mm)	40.1 $\pm$ 6.4
LVMWT (mm)	19.2 $\pm$ 5.3
LVEF (%)	68.2 $\pm$ 11.1
Conventional risk factors	
Family history of SCD, n (%)	13 (15.9)
Unexplained syncope, n (%)	6 (7.3)
History of VF/VT, n (%)	9 (11.0)
NSVT, n (%)	10 (12.2)
LVMWT >30 mm, n (%)	5 (6.1)
Toronto score	0.4 $\pm$ 5.3
CMR imaging	
LGE-positive, n (%)	60 (73.2)
LGE extent (%)	9.91 $\pm$ 10.4
No. of segments with LGE	2.5 $\pm$ 2.8

AF, atrial fibrillation; BNP, B-type natriuretic peptide; CMR, cardiac magnetic resonance; HCM, hypertrophic cardiomyopathy; HOcm, hypertrophic obstructive cardiomyopathy; LAD, left atrium diameter; LGE, late gadolinium enhancement; LVEF, left ventricular ejection fraction; LVMWT, left ventricular maximal wall thickness; NSVT, non-sustained ventricular tachycardia; SCD, sudden cardiac death; VF, ventricular fibrillation; VT, ventricular tachycardia.

Figure 2. Causative mutations in the studied patients with hypertrophic cardiomyopathy (n=82). Single mutations were detected in 51.2% of the study patients. Among these causative mutations, major components were mutations in the MYBPC3, TNNI3, and MYH7 genes. Multiple mutations were detected in 2.4% of patients. However, the status of mutations in 46.4% of patients remained undetermined.

Table 2. Potentially Causative Mutations in the Study Patients With HCM Classified According to HGMD Registration and ClinVar Database

Case no.	Gene	Chr.	Exon	Position (build 37)	DNA change	Protein change
HCM-001, 190, 208, 228, 373, 501, 557, 607, 625, 645, 667, 680, 685	TNNI3	19	7	55665397	c.547_549delAAG	p.Lys183del
HCM-018	MYL3	3	3	46902192	c.281G>A	p.Arg94His
HCM-031	MYBPC3	11	4	47371592	c.478C>T	p.Arg160Trp
HCM-038	MYBPC3	11	18	47362715	c.1871A>C	p.Asn624Thr
HCM-076	TPM1	15	2	63336299	c.188C>T	p.Ala63Val
HCM-300, 539, 616	MYBPC3	11	23	47360094	c.2285T>A	p.Val762Asp
HCM-425, 567	MYBPC3	11	26	47356663	c.2833_2834delICG	p.Arg945GlyfsTer105
HCM-526	MYBPC3	11	25	47359085	c.2459G>A	p.Arg820Gln
HCM-529	MYH7	14	5	23901997	c.353C>T	p.Ser118Leu
HCM-534, 586, 617	MYBPC3	11	12	47367848	c.1000G>A	p.Glu334Lys
HCM-546	MYH7	14	36	23884594	c.5279C>G	p.Thr1760Arg
HCM-552	MYH7	14	23	23893274	c.2761_2763delGAG	p.Glu921del
HCM-565	MYBPC3	11	13	47365110	c.1156G>T	p.Glu386Ter
	MYBPC3	11	23	47360104	c.2275G>A	p.Glu759Lys
HCM-561	JPH2	20	4	42744802	c.1513G>A	p.Gly505Ser
HCM-580	MYBPC3	11	–	47369974	c.772+1G>A	–
HCM-588	MYH7	14	27	23889157	c.3623A>G	p.Asp1208Gly
HCM-591	MYH7	14	25	23891464	c.3170G>A	p.Gly1057Asp
HCM-592	TNNT2	1	12	201332507	c.517G>A	p.Glu163Lys
HCM-601	MYH7	14	16	23896866	c.1816G>A	p.Val606Met
HCM-602	MYL2	12	3	111352091	c.173G>A	p.Arg58Gln
HCM-610	MYBPC3	11	29	47355161	c.3137C>T	p.Thr1046Met
	TPM1	15	2	63336299	c.188C>T	p.Ala63Val
HCM-611	MYH7	14	14	23898244	c.1327A>G	p.Ile443Val
HCM-623	MYH7	14	20	23894983	c.2207T>C	p.Ile736Thr
HCM-650	MYBPC3	11	–	47354886	c.3191–2A>C	–
HCM-677	MYH7	14	23	23893235	c.2803G>A	p.Glu935Lys
HCM-681	MYBPC3	11	27	47356593	c.2905C>T	p.Gln969Ter
HCM-690	MYBPC3	11	14	47364429	c.1409G>A	p.Arg470Gln

SIFT scores (ranging from 0 to 1) were calculated by SIFT version 5.2.2; a SIFT score <0.05 considered as deleterious. PolyPhen-2 scores (ranging from 0 to 1) were calculated by PolyPhen-2 version 2.2.2; a PolyPhen-2 score >0.85 considered as probably damaging. The cutoff of CADD score on deleteriousness to identify potentially pathogenic variants was 20. CADD, Combined Annotation Dependent Depletion; Chr, chromosome; DM, damaging mutation; HCM, hypertrophic cardiomyopathy; Het, heterozygous; HGMD, Human Gene Mutation Database; PolyPhen-2, Polymorphism Phenotyping v2 Score; Position, nucleotide position; SIFT, Sorting Intolerant from Tolerant.

(Table 2 continued the next page.)

Genotype	MAF (%)	SIFT	PolyPhen-2	CADD score	Variant category
Het	0.00	–	–	Potentially pathogenic (22.4)	DM ²¹
Het	0.00	Deleterious (0.02)	Benign (0.009)	Potentially pathogenic (25.5)	DM ¹⁵
Het	0.00	Deleterious (0)	Probably damaging (0.993)	Potentially pathogenic (28.8)	DM ³⁶
Het	0.00	Tolerated (0.27)	Benign (0.259)	Potentially pathogenic (23.4)	Not listed
Het	0.00	Tolerated (0.11)	Benign (0.034)	Potentially pathogenic (22.9)	DM ³⁷
Het	0.00	Deleterious (0)	Possibly damaging (0.893)	Potentially pathogenic (29.0)	DM ¹⁸
Het	0.00	–	–	Not indicated (16.9)	DM ³⁶
Het	0.00	Deleterious (0.01)	Probably damaging (0.954)	Potentially pathogenic (35.0)	DM ³⁶
Het	0.00	Deleterious (0)	Benign (0.37)	Potentially pathogenic (28.1)	Not listed
Het	0.00	Tolerated (0.05)	Benign (0.344)	Potentially pathogenic (24.9)	DM ³⁶
Het	0.00	Deleterious (0.01)	Probably damaging (1)	Potentially pathogenic (29.7)	Not listed
Het	0.00	–	–	Potentially pathogenic (22.1)	Not listed
Het	0.00	–	–	Potentially pathogenic (38.0)	Not listed
Het	0.00	Deleterious (0.03)	Probably damaging (0.956)	Potentially pathogenic (35.0)	Not listed
Het	0.00	Tolerated (0.71)	Benign (0.003)	Not indicated (5.0)	DM ³⁸
Het	0.00	–	–	Potentially pathogenic (26.4)	Not listed
Het	0.00	Deleterious (0)	Probably damaging (1)	Potentially pathogenic (27.3)	Not listed
Het	0.00	Deleterious (0.03)	Probably damaging (0.999)	Potentially pathogenic (22.4)	DM ³⁹
Het	0.00	–	–	Potentially pathogenic (34.0)	DM ⁴⁰
Het	0.00	Deleterious (0)	Probably damaging (0.907)	Potentially pathogenic (26.8)	DM ⁴¹
Het	0.00	Tolerated (0.16)	Probably damaging (0.995)	Potentially pathogenic (27.7)	DM ⁴²
Het	0.00	Tolerated (0.06)	Benign (0.044)	Potentially pathogenic (18.6)	DM ³⁶
Het	0.00	Tolerated (0.11)	Benign (0.034)	Potentially pathogenic (22.9)	DM ³⁷
Het	0.00	Tolerated (0.25)	Probably damaging (0.99)	Potentially pathogenic (22.2)	Not listed
Het	0.00	Tolerated (0.1)	Probably damaging (0.999)	Potentially pathogenic (24.7)	DM ⁴³
Het	0.00	–	–	Potentially pathogenic (24.0)	Not listed
Het	0.00	Deleterious (0.04)	Probably damaging (0.948)	Potentially pathogenic (33.0)	DM ⁴⁴
Het	0.00	–	–	Potentially pathogenic (38.0)	DM ⁴⁵
Het	0.00	–	–	Potentially pathogenic (34.0)	DM ⁴⁶

Table 3. Logistic Regression Analysis of Clinical Findings in Predicting the Mutation-Positive Status of Patients With HCM (n=82)

Variable	Univariate			Multivariate					
	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value
Age	0.97	0.94–0.99	0.02*						
Male	0.42	0.17–1.03	0.06	0.03	0.0006–0.41	0.028*			
Familial	16.7	5.08–77.6	0.00003*	7.32	0.91–100.5	0.082	8.01	0.99–112.2	0.072
AF	0.85	0.19–3.84	0.83						
BNP	1.0	0.99–1.0	0.591						
Fragmented QRS	2.53	1.05–6.34	0.042*						
HOCM	1.57	0.52–5.07	0.431						
LAD	0.97	0.9–1.03	0.319						
LVMWT	1.06	0.98–1.17	0.172						
LVEF	0.99	0.95–1.03	0.618						
Family history of SCD	6.0	1.47–40.7	0.026*						
VF/VT	8.22	1.41–156.5	0.052						
NSVT	2.21	0.56–10.9	0.278						
Toronto score	1.17	1.07–1.31	0.0024*				1.11	0.96–1.54	0.166
%LGE on CMR	1.77	1.42–2.46	0.00002*	2.12	1.51–3.83	0.0009*	1.75	1.38–1.54	0.0002*

*P<0.05. OR, odds ratio. Other abbreviations as in Table 1.

Differences between mutation-positive patients within each %LGE category were analyzed using the Cochran-Armitage test to assess the trend of proportions.

Ethical Considerations

The Ethics Committee of Kanazawa University approved this study. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Written informed consent was given by all participants or their guardians.

Results

Characteristics of the Study Patients

The clinical characteristics of the study patients are listed in **Table 1**: 82 unrelated patients (47 men, mean age=55.4 years, familial cases=29) with clinically affected HCM were evaluated. The mean LVMWT was 19.2 mm. CMR analysis showed that 60 patients had LGE in a total of 205 segments (2.5±2.8 segments/patient) and the average %LGE was 9.91%. The measurement protocol of %LGE reached a sufficiently high intra- and interobserver reproducibility (Lin's concordance correlation coefficient=0.95 and 0.91, respectively).

Panel-Wide Sequencing

Targeted sequencing was performed in 82 HCM patients. In the 15 patients using our original 21-gene panel, the mean read length was 149.2 base pairs, the mean sequencing coverage depth was 379.4× per base across the target genes, and the coverage rate for the target coding lesions (20×) was 97.6%. In the 67 patients using the TruSight Cardio panel, the mean read length was 278.4 base pairs, the mean sequencing coverage depth was 456.2×, and the coverage rate for the target coding lesions (20×) was 99.8%. A single mutation was detected in 51.2% of the studied patients with HCM (**Figure 2**). Among these causative mutations, major components were mutations in the MYBPC3 gene

(n=15), the TNNI3 gene (n=13), and the MYH7 gene (n=9). Multiple mutations were detected in 2.4% of all patients. However, the status of mutations in 46.4% of the patients remained undetermined.

Detailed information about potentially causative mutations is listed in **Table 2** according to evidence-based assessment or in silico prediction. Each mutation was confirmed by Sanger sequencing. A representative case is shown in **Figure S1**. The proband, a 49-year-old man with clinically affected HCM, showed LGE confined to the basal anteroseptal wall on CMR in a pattern typical for HCM, but LVH was not as prominent (LVMWT=15.1 mm). Panel-wide sequencing revealed a pathogenic heterozygous p.Lys183del mutation in the TNNI3 exon 7,²¹ which was confirmed by Sanger sequencing.

Correlations Between Presence of Mutations and Clinical Parameters in HCM

We first assessed whether LGE on CMR is an independent factor causing an increase in the pre-genetic test probability of mutation-positive HCM. Multivariate logistic regression analyses revealed that the %LGE on CMR was independently associated with the presence of mutations in patients with clinically affected HCM after adjustments for sex and familial situation (odds ratio [OR], 2.12; 95% confidence interval [CI], 1.51–3.83; P=0.0009; model 1 in **Table 3**) as the most parsimonious model based on Akaike's Information Criterion. As a result of model 2, which added a Toronto score integrating conventional risk factors such as younger age, female sex, and LV morphology for mutation-positive HCM into model 1, the %LGE still remained an independent predictor for the presence of sarcomere mutations (OR, 1.8; 95% CI, 1.4–1.5; P=0.0002; model 2 in **Table 3**).

ROC plots of %LGE demonstrated an extremely high AUC for mutation-positive HCM (AUC=0.96) (**Figure 3**). When the cutoff value was set at 8.1%, the sensitivity, specificity, PPV, and NPV of %LGE were 93.2%, 89.5%, 91.1%, and 91.9%, respectively. The AUC of %LGE was greater than that of the Toronto genetic risk score

(AUC=0.69, $P=0.00002$), which is reported as an accurate predictive tool for mutation-positive HCM.⁸ The AUC of the %LGE was greater than that of LGE-positive status (AUC=0.79, $P=0.00008$).

Correlations between the extent of LGE and mutation-positive or -negative status are shown in representative HCM patients (Figure 1). A 54-year-old male with HCM, who had a family history of sudden cardiac death, showed asymmetric septal hypertrophy (17 mm) and 13.1% LGE in the anteroseptal wall on CMR. Panel sequencing detected a relatively large deletion in exon 26 of MYBPC3 (Figure 1D). This variant is reported as a causative mutation for HCM (Table 2). On the other hand, a 64-year-old male with moderate LVH predominantly in the inferior wall (LVMWT=25 mm) did not show LGE on CMR. Panel sequencing did not detect any pathogenic mutations (Figure 1E).

We then investigated whether the rate of detection of mutation-positive HCM using genetic testing could be improved according to increased extent of LGE. The proportion of mutation carriers significantly increased with increasing %LGE ($P<0.01$ for trends, Figure 4); 91% of patients with LGE $\geq 8.1\%$ had disease-causing mutations. Conversely, among 37 patients with LGE $<8.1\%$, only 3 were carriers of a pathogenic mutation (8.1%). Together, these findings suggested that the extent of LGE in clinically affected HCM patients strongly increases the pre-test probability of patients having a disease-causing mutation.

Discussion

The present study, which focused on the genetic basis of myocardial scarring in HCM, made 3 major findings: (1) the extent of a myocardial scar determined by LGE on CMR was an independent factor for predicting mutation-positive HCM (OR 2.12 [95% CI 1.51–3.83], $P<0.01$); (2) the proportion of mutation-positive HCM increased linearly relative to %LGE ($P<0.01$ for trends); and (3) LGE (cutoff $>8.1\%$) strongly discriminated mutation-positive

Figure 3. Receiver-operating characteristic (ROC) curve of predictive indices and cutoff value for prediction of mutation-positive status in patients with hypertrophic cardiomyopathy (n=82). ROC plots of %LGE demonstrate an excellent area under the curve (AUC) for the mutation-positive status (AUC=0.96). Sensitivity, specificity, positive, and negative predictive values of %LGE were 93.2%, 89.5%, 91.1%, and 91.9%, respectively, when the cutoff value was set at 8.1%. The AUC of %LGE was greater than that of the Toronto genetic risk score (AUC=0.69, $P<0.01$).

Figure 4. Frequency of mutation-positive hypertrophic cardiomyopathy (HCM) at different extents of late gadolinium enhancement (LGE). Bar graph shows the presence of mutations according to the extent of LGE. Causative mutation for HCM was present predominantly in the group with a high %LGE. $P<0.001$ for trends was obtained for all comparisons across %LGE categories.

from mutation-negative HCM in a clinically affected HCM population. These results demonstrated the close relationship between the presence of sarcomere gene mutations and the extent of LGE.

Myocardial Scarring as a Primary Expression of HCM

Myocardial scarring, often referred to as replacement fibrosis, is assumed to occur subsequent to cardiac muscle cell disorganization, even in the absence of epicardial coronary artery disease.^{6,22} Recent data indicated that interstitial fibrosis may represent a primary phenotypic expression of HCM and is not necessarily a time-related feature.^{23,24} Several studies have shown early activation of profibrotic genetic pathways in mice models with sarcomere gene mutations.^{25,26} Thus, it is considered that myocardial scars occur as a result of focal myocardial damage in the hypertrophied myocardium whereas interstitial fibrosis is primarily induced by sarcomere gene mutations in HCM.

Intriguingly, in this study, NGS enabled us to reveal direct associations between myocardial scars and sarcomere gene mutations in HCM; LGE was the only independent factor that could predict the identification of sarcomere gene mutations, even after adjusting for age, sex, familial situation, and the extent of LVMWT (multivariate regression model 2 in **Table 3**). These findings suggested that myocardial scars, when determined by LGE, might be directly induced by a sarcomere gene mutation, irrespective of the extent of LVMWT. This hypothesis may be supported by previous clinical and experimental studies. HCM patients show a greater extent of myocardial scarring than patients with LVH caused by pressure overload such as hypertension or aortic stenosis.²⁷ The physiological basis of LGE is mainly based on prolonged washout of the contrast agent, which is related to decreased capillary density within the fibrotic myocardial tissue. Moreover, myocytes with sarcomere gene mutations leads to expansion of the interstitial matrix, which may in turn lead to increased contrast agent retained within fibrotic tissue because of the decreased capillary density in the activation cascade of myocyte enhancer factor-2.²⁸ An *in vivo* animal study demonstrated that myosin regulatory light chain transgenic HCM mice exhibited myocardial scarring even before the development of LVH.⁷ Although the mechanism by which sarcomere gene mutations lead to myocardial scarring remains unclear, severely narrowed small coronary arteries, a characteristic feature of HCM, also may be directly caused by the sarcomere gene mutations, partly explaining this association.^{12,29} Further studies are needed to determine the pathogenesis of small coronary artery disease in HCM.

Novel Interpretation of the Extent of LGE Based on Molecular Diagnosis of HCM

Although LGE has been studied as a tissue characterization technique in HCM,^{4,30} it is often challenging to obtain quantitative quality LGE imaging in HCM patients, because of the lack of a universally accepted protocol for acquisition and evaluation, difficulty in defining the reference area, pathophysiological diversity of the disease, and mechanical and human conditions at the time of image acquisition. When we analyzed delayed-enhancement images at 6SD as previously reported,^{12,31} there were strong linear correlations between observations and observers in the measurement of %LGE ($r \geq 0.9$ for 2 comparisons in **Figure S2**). Reproducibility was supported by good intra-

and interobserver correlations between %LGE (Lin's concordance correlation coefficient=0.95 and 0.91, respectively).

In previous CMR studies, variation in the extent of LGE was considered to reflect heterogeneity of HCM phenotypes.¹² However, our data suggested that HCM with an increased extent of LGE may be genetically different from HCM with absence or a low extent of LGE, even though these two groups are indistinguishable based on LV morphology. A recent retrospective cohort study revealed that a subgroup without a family history or sarcomere gene mutations should be classified as a previously unrecognized subgroup known as "nonfamilial HCM" because of its prognostic features with later onset and less risk of major cardiac events.⁸ Thus, clinically affected HCM patients exhibiting either none or only a small extent of LGE could be classified into the subgroup with different underlying pathogenesis.

These observations may also have an effect on strategies of risk stratification of HCM. Previous data showed that HCM with none or a small extent of LGE is associated with favorable clinical outcomes.³² At the same time, HCM patients who do not have a sarcomere gene mutation may show better prognosis than those harboring such mutations.^{8,33,34} Because our study revealed direct associations between the extent of LGE and a sarcomere gene mutation, advanced expertise from the ongoing HCMR study (NCT 01915615)³⁵ is anticipated to clarify the long-term clinical course in HCM, specifically in the previously unrecognized subgroup of mutation-negative HCM without significant LGE.

Study Limitations

Several limitations remain in this study. First, pathogenic mutations within those genes not included in the panels may have been missed. However, our gene panel covered sarcomere genes and metabolic cascade-related genes, indicating that known HCM-causing genes might not be associated with cases of LVH in which disease-causing mutations were not identified in this study. Second, large deletions might not be detected by NGS. However, this possibility is quite low because we carefully checked the average read length and coverage depth in each sample. Additional whole-exome sequencing or whole-genome sequencing is warranted to investigate the nature of LVH related to unknown genes in HCM patients without sarcomere mutations. Third, our data did not include evaluation of T1 mapping, which is sensitive to detecting fibrotic areas, because of technical limitations in the initial cases.

Conclusions

LGE on CMR was independently associated with the presence of mutations in clinically affected HCM patients. HCM with increased myocardial scarring may be genetically different from HCM with little or no extent of myocardial scarring.

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Conflict of Interest / Sources of Funding

None.

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Supplementary Files

Supplementary File 1

Figure S1. Clinical and genetic profiles of a representative case with HCM.

Figure S2. Scatterplots of intra- and interobserver variations for %LGE measurements.

Table S1. The 21 genes comprising the original panel and 174 genes comprising the TruSight Cardio panel

Please find supplementary file(s);

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