A Validation Study of the Mayo Clinic Phenotype-Based Genetic Test Prediction Score for Japanese Patients With Hypertrophic Cardiomyopathy

Toshihiro Moriki, MD; Toru Kubo, MD; Kenta Sugiura, MD; Yuri Ochi, MD;
Yuichi Baba, MD; Takayoshi Hirota, MD; Naohito Yamasaki, MD;
Akinori Kimura, MD; Yoshinori L. Doi, MD; Hiroaki Kitaoka, MD

Background: Hypertrophic cardiomyopathy (HCM) is a primary myocardial disorder with an autosomal-dominant disorder mainly caused by mutations in sarcomere genes. Recently, a phenotype-based genetic test prediction score for patients with HCM was introduced by Mayo Clinic. The genotype score was derived on the basis of the predictive effect of 6 clinical markers, and the total score was shown to be correlated with the yield of genetic testing. However, it has not been determined whether this prediction model is useful in Japanese HCM patients.

Methods and Results: The utility of the Mayo Clinic HCM genotype predictor score in 209 Japanese unrelated patients with a clinical diagnosis of HCM who had undergone genetic testing for 6 sarcomere genes was assessed. Overall, 55 patients (26%) had pathogenic or likely pathogenic variants (60% being genotype-positive in familial cases). We divided the patients into 6 groups (groups with scores of from –1 to 5) according to the prediction score. The yields of genetic testing in the groups with scores of –1, 0, 1, 2, 3, 4, and 5 were 8%, 16%, 24%, 48%, 50%, 100%, and 89%, respectively, with an incremental increase in yield between each of the score subgroups ($P<0.001$).

Conclusions: The Mayo Clinic HCM genotype predictor score is useful for predicting a positive genetic test result in Japanese HCM Patients.

Key Words: Hypertrophic cardiomyopathy; Mayo HCM genotype predictor score; Phenotype; Sarcomere mutations

Hypertrophic cardiomyopathy (HCM) is a primary myocardial disorder with a broad spectrum of clinical features.^{1–3} HCM is the most common hereditary cardiomyopathy in the world, with a prevalence of >1 in 500 people. It is an autosomal-dominant trait disease that is mainly caused by mutation in the gene encoding sarcomere proteins.^{4,5} The clinical course of HCM patients with a sarcomere gene mutation has been reported to be significantly worse than that of HCM patients without a mutation.^{6–9} Recently, an applicable phenotype-derived score that provides a pretest probability for a positive HCM genetic test result was introduced by the Mayo Clinic.¹⁰ The investigators from the Mayo Clinic determined the predictive effect of 5 clinical markers that independently predicted a positive genetic test result, as well as 1 negative predictor. However, some clinical features, such as apical HCM, in Japanese patients differ

Editorial p 675

from those in Western patients.

The aim of this study was to validate and determine whether the Mayo Clinic phenotype-based genetic test prediction score is useful in Japanese HCM patients.

Methods

Study Design and Subjects

This study was a retrospective study to validate the scoring system provided by the Mayo Clinic for determining the likelihood of a positive HCM genetic test. We analyzed data for 209 consecutive unrelated patients with HCM who had agreed to undergo genetic testing between March 2003 and December 2015. All of the patients were evalu-

Received August 25, 2020; revised manuscript received November 19, 2020; accepted November 21, 2020; J-STAGE Advance Publication released online January 23, 2021 Time for primary review: 12 days

Department of Cardiology and Geriatrics, Kochi Medical School, Kochi University, Kochi (T.M., T.K., K.S., Y.O., Y.B., T.H., N.Y., Y.L.D., H.K.); Department of Molecular Pathogenesis, Medical Research Institute, Tokyo Medical and Dental University, Tokyo (A.K.), Japan

Mailing address: Toru Kubo, MD, Department of Cardiology and Geriatrics, Kochi Medical School, Kochi University, Oko-cho, Nankoku, Kochi 783-8505, Japan. E-mail: jm-kubotoru@kochi-u.ac.jp

All rights are reserved to the Japanese Circulation Society. For permissions, please e-mail: cj@j-circ.or.jp
ISSN-1346-9843

Table 1. Clinical Characteristics of the 209 Unrelated Patients With HCM at Initial Evaluation

Age at diagnosis, years	56±16
Age at diagnosis <45 years, n (%)	50 (24)
Gender: men, n (%)	146 (70)
Family history of HCM, n (%)	48 (23)
Family history of sudden cardiac death, n (%)	30 (14)
History of hypertension, n (%)	94 (45)
Echocardiographic data	
Subtype, n (%)	
HOCM	36 (17)
MVO	9 (4)
D-HCM	6 (3)
Apical HCM	38 (18)
HNCM	120 (57)
Maximum LV wall thickness, mm	19±4
Maximum LV wall thickness ≥20 mm, n (%)	102 (49)
Reverse-curve HCM, n (%)	25 (12)

D-HCM, dilated phase of hypertrophic cardiomyopathy; HCM, hypertrophic cardiomyopathy; HNCM, hypertrophic non-obstructive cardiomyopathy without obstruction, other than for D-HCM and apical HCM; HOCM, hypertrophic obstructive cardiomyopathy; LV, left ventricular; MVO, midventricular obstruction.

ated at Kochi Medical School Hospital for confirming diagnosis, risk assessment, and symptom management. The diagnosis of HCM was based on echocardiographic demonstration of unexplained left ventricular hypertrophy (i.e., maximum LV wall thickness ≥15 mm) in the absence of other cardiac diseases that could produce hypertrophy of such magnitude (e.g., arterial hypertension, aortic stenosis or storage disease; Fabry disease was excluded by measurements of plasma α -galactosidase A activity in male patients, and other storage diseases were also excluded by confirming extracardiac findings suggestive of secondary cardiomyopathies as much as possible). The study was approved by the Ethics Committee on Medical Research of Kochi Medical School and followed the Declaration of Helsinki and the ethical standards of the responsible committee on human experimentation. Written informed consent was given from all of the patients or their parents in accordance with the guidelines of the Ethics Committee on Medical Research of Kochi Medical School. Patients' data including medical history and echocardiographic data were collected from medical records. Based on morphologic and hemodynamic assessments by echocardiography, we divided the patients into the following 5 groups: (1) hypertrophic obstructive cardiomyopathy (HOCM), defined as the presence of basal LV outflow tract (LVOT) obstruction (gradient ≥30 mmHg at rest); (2) midventricular obstruction (MVO), defined as the presence of systolic LV cavity obliteration at the mid-ventricle creating MVO with a peak systolic gradient ≥30 mmHg at rest; (3) dilated phase of HCM (D-HCM), defined as LV systolic dysfunction of global ejection fraction (EF) <50% (global EF was determined by using the modified Simpson's method.) with unexplained hypertrophied LV (maximum LV wall thickness ≥15 mm) or previous documentation of unexplained LVH on echocardiography (maximum LV wall thickness ≥15 mm); (4) apical HCM, defined as hypertrophy confined to the LV apex; and (5) hypertrophic non-obstructive cardiomyopathy (HNCM): HCM without obstruction other

than D-HCM and apical HCM. A reverse curve septal shape on echocardiography was defined as a predominant mid-septal convexity toward the LV cavity, with the cavity itself having an overall crescent shape, as previously described.¹¹ This morphology was distinguished from the sigmoid septal subtype, the apical hypertrophy subtype and the neutral septal contour subtype.

Genetic Analysis

In this investigation, we screened for mutations in the protein-coding exons of 6 sarcomere genes encoding cardiac myosin-binding protein C (*MYBPC3*), cardiac β myosin heavy chain (*MYH7*), cardiac troponin T (*TNNI2*), cardiac troponin I (*TNNI3*), α tropomyosin (*TPMI*), and α actin (*ACTC*). After written informed consent had been given, peripheral blood samples were taken from the patients. Then, deoxyribonucleic acid (DNA) was extracted. In vitro amplification of all exons was performed by polymerase chain reaction using oligonucleotide primers. Information on primer sequences is available in our previous paper.⁹ Sequencing was performed by using a BigDye Terminator Cycle Sequencing Kit from Applied Biosystems Inc. (no. 4336774; Foster City, CA, USA). The sequences were analyzed on an ABI PRISM 3100-Avant Genetic Analyzer in accordance with the manufacturer's instructions. In patients in whom a mutation was identified, confirmation was obtained by re-analysis with direct sequencing from a second blood sample.

Genetic Diagnosis

After the genetic analysis, we defined variants as pathogenic or likely pathogenic variants using the following steps. At first, we selected variants that were absent in a normal healthy control group of 100 subjects in Kochi Prefecture. Second, because a homozygous variant was not detected in our genetic analysis, we selected rare variants, which were defined as variants with a minor allele frequency <0.01 for autosomal dominant heterozygous variants in genetic databases including the Exome Aggregation Consortium (ExAC) browser (<http://exac.broadinstitute.org>) and Human Genetic Variation Database (HGVD) (<http://www.hgvd.genome.med.kyoto-u.ac.jp>). Then, we excluded variants that were described as "benign" or "likely benign" in an online database of human genotypes and phenotypes (ClinVar; <http://www.ncbi.nlm.nih.gov/clinvar/>). We also excluded variants that were confirmed as non-segregation with the disease in the family. Finally, in this study, we used the variants judged as pathogenic or likely pathogenic variants in the interpretation of sequence variants recommended by the 2015 American College of Medical Genetics and Genomics (ACMG) standards and guidelines.¹²

Validation of the Mayo Clinic Phenotype-Based Genetic Test Prediction Score

In order to evaluate the predictive value of the Mayo Clinic HCM genotype predictor score, all patients were checked for the following 6 factors.¹⁰ The scoring system assigned 1 point for the presence of each of these established variables: 'age at diagnosis <45 years', 'maximum LV wall thickness ≥20 mm', 'positive family history of HCM', 'positive family history of sudden cardiac death', and 'reverse curve septal shape on echocardiography'. The 'presence of hypertension' resulted in subtracting 1 point from their score. The patients were divided into 6 groups

Gene	Nucleotide change	Amino acid change	Variant type	Pathogenicity by the 2015 ACMG guidelines
MYBPC3	c.318delT	p.Pro106fs	Frameshift	Pathogenic
MYBPC3	c.890C>G	p.Ser297Ter	Stop gained	Pathogenic
MYBPC3	c.1112C>T	p.Pro371Leu	Missense	Likely pathogenic
MYBPC3	c.1597delC	p.Gln533fs	Frameshift	Pathogenic
MYBPC3	c.1777delT	p.Ser593fs	Frameshift	Pathogenic
MYBPC3	c.2285T>A	p.Val762Asp	Missense	Likely pathogenic
MYBPC3	c.2459G>A	p.Arg820Gln	Missense	Pathogenic
MYBPC3	c.2833_2834delCG	p.Arg945fs	Frameshift	Pathogenic
MYBPC3	c.3412C>T	p.Arg1138Cys	Missense	Likely pathogenic
MYH7	c.428G>A	p.Arg143Gln	Missense	Pathogenic
MYH7	c.727C>T	p.Arg243Cys	Missense	Likely pathogenic
MYH7	c.1357C>T	p.Arg453Cys	Missense	Pathogenic
MYH7	c.1686C>A	p.Asn562Lys	Missense	Likely pathogenic
MYH7	c.1987C>T	p.Arg663Cys	Missense	Pathogenic
MYH7	c.2605C>T	p.Arg869Cys	Missense	Likely pathogenic
MYH7	c.2803G>A	p.Glu935Lys	Missense	Likely pathogenic
MYH7	c.4259G>A	p.Arg1420Gln	Missense	Likely pathogenic
TNNI3	c.484C>T	p.Arg162Trp	Missense	Pathogenic

ACMG, American College of Medical Genetics and Genomics; MYBPC3, cardiac myosin-binding protein C gene; MYH7, cardiac β myosin heavy chain gene; TNNI3, cardiac troponin I gene.

Marker	Genetic test (+) (n=55)	Genetic test (–) (n=154)	P value
Age at diagnosis <45 years	22 (40)	28 (18)	0.001
Maximum LVWT $\geq$ 20 mm	27 (49)	75 (49)	0.960
Family history of HCM	29 (53)	19 (12)	<0.001
Family history of SCD	14 (25)	16 (10)	0.006
Reverse-curve HCM	13 (24)	12 (8)	0.002
History of hypertension	13 (24)	81 (53)	<0.001

Data are presented as n (%). HCM, hypertrophic cardiomyopathy; LVWT, left ventricular wall thickness; SCD, sudden cardiac death.

by total points (groups with scores of –1 to 5), as shown in our original paper.¹⁰

Statistical Analysis

All data are expressed as mean $\pm$ SD or frequency (percentage). Comparisons of age at diagnosis and maximum LV wall thickness between genotype-positive and genotype-negative patients were assessed by using Student's t-test for normally distributed variables. Pearson's chi-squared test was used for comparisons between categorical variables, and Fisher's exact test was used when expected frequency was lower than 5. Multivariate logistic regression analysis was performed to estimate the odds ratios for clinical markers of a positive genetic test result. The Cochran-Armitage trend test was used to evaluate the presence of a significant trend in correlation between genotype score and yield of genetic testing. Statistical analysis was performed using IBM SPSS version 21.0 software (IBM Corp., Armonk, NY, USA). Probability values <0.05 were considered statistically significant.

Results

Baseline Clinical Characteristics of Patients in Our HCM Population

Clinical characteristics of the 209 unrelated HCM patients in our cohort at initial evaluation are summarized in **Table 1**. The age at diagnosis was 56 ± 16 (range: 6–87) years, and 146 patients (70%) were men. Fifty patients (24%) were diagnosed with HCM at age <45 years. Forty-eight patients (23%) had a family history of HCM and 30 patients (14%) had a family history of sudden cardiac death. Mild hypertension was observed in 94 patients (45%). Of the 209 patients, there were 36 patients in the HOCM group, 9 patients in the MVO group, 6 patients in the D-HCM group, 38 patients in the apical HCM group, and 120 patients in the HNCM group. Maximum LV wall thickness was 19 ± 4 mm, and 102 patients (49%) had a maximum LV wall thickness ≥ 20 mm. A reverse curve shape was observed in 25 patients (12%).

Genetic Results

The identified pathogenic or likely pathogenic variants are

Figure 1. Distribution of the parameters of age at diagnosis (A) and maximum LV wall thickness (B) between genetic test-positive and genetic test-negative patients.

Table 4. Multivariate Analysis of the Clinical Markers of a Positive Genetic Test Result			
Markers of the Mayo genotype predictor score	Odds ratio	95% CI	P value
Age at diagnosis <45 years	1.374	0.616–3.065	0.437
Maximum LVWT ≥20 mm	0.798	0.390–1.629	0.535
Family history of HCM	5.183	2.376–11.304	<0.001
Family history of SCD	1.566	0.597–4.104	0.362
Reverse-curve HCM	2.399	0.872–6.605	0.090
History of hypertension	2.010	0.917–4.404	0.081

CI, confidence interval; HCM, hypertrophic cardiomyopathy; LVWT, left ventricular wall thickness; SCD, sudden cardiac death.

summarized in **Table 2** (detailed information on the variants is provided in **Supplementary Table 1**). Overall, 55 patients (26%) were genotype-positive. Among the 48 patients with a family history of HCM, the prevalence of sarcomere gene variants was 60% (29 of 48 familial HCM patients). Sarcomere gene variants were detected in 8 (22%) of the 36 HOCM patients, 3 (33%) of the 9 MVO patients, 5 (83%) of the 6 D-HCM patients, 2 (5%) of the 38 apical patients, and 37 (31%) of the 120 HNCM patients.

Table 3 shows the 6 clinical markers of the Mayo Clinic phenotype-based genetic test prediction score and comparison between genotype-positive and genotype-negative patients from the univariate analysis. All markers except ‘a maximum LV wall thickness ≥20 mm’ were significantly different between the 2 groups. **Figure 1** shows the distribution of the parameters of age at diagnosis and maximum LV wall thickness between genetic test-positive and genetic test-negative patients. Regarding age at diagnosis, patients with genotype positive were significantly younger than patients with genotype negative (51.9±15.6 vs. 58.0±15.3 years) (**Figure 1A**). In contrast, there was no difference in maximum LV wall thickness between the 2 groups (19.5±3.8 vs. 20.0±3.8 years) (**Figure 1B**). As shown in **Table 4**, multivariate analysis found the odds ratio of each clinical marker in Japanese HCM patients.

Validation of the Mayo Clinic Phenotype-Based Genetic Test Prediction Score

Figure 2 shows the yield of genetic testing in each score subgroup in our validation cohort and the original derivation cohort. Regarding the patients distribution of total prediction scores, a score of 0 or 1 was most frequent (**Figure 2** and **Supplementary Table 2**). In our cohort, the yield of genetic testing ranged from 8% for patients with −1 point to more than 80% for patients with 4 or 5 points, with an incremental increase in yield between each of the score subgroups (P<0.001).

Discussion

We investigated the validity of the Mayo Clinic phenotype-based genetic test prediction score for predicting a positive genetic test result in 209 unrelated HCM patients, and we found that there was a significant trend for an association of a higher phenotype-based genetic prediction score with the likelihood of a positive genetic test result in our Japanese HCM population. At first glance, the group with a score of 4 seemed to have a higher rate of genotype-positive patients than the group with a score of 5, but it was thought to be caused by the low number of patients.

HCM is a primary myocardial disorder with heterogeneous morphologic, functional, and clinical features.^{1–3}

Figure 2. Yield of genetic testing in each score subgroup in our validation cohort (Kochi cohort) and in the original derivation cohort (Mayo Clinic cohort). *Adapted from Bos et al¹⁰ with permission.

Molecular genetic studies have shown that HCM is mainly caused by single heterozygous mutations in genes for sarcomere proteins.^{1–5} The Japanese guidelines emphasize that when cardiomyopathies are suspected, careful consideration of the genetic background is needed for diagnosis and management of cardiomyopathies.¹³ Although a specific therapeutic strategy based on the genotype in HCM patients has not yet been established, a positive genetic test result has several merits such as confirmation of the disease etiology, use for a family survey, and prediction of disease severity.^{1–3} However, the yield of genetic testing for sarcomere genes in patients with a clinical diagnosis of HCM ranges from only 30% to 50%.^{1–3,14} Recently, the Mayo Clinic phenotype-based genetic test prediction score was introduced to provide a prior yield of the HCM genetic test.¹⁰ Afterwards, the investigators in the Mayo Clinic were able to successfully validate the Mayo Clinic prediction score in their new HCM cohort, demonstrating a similar increased yield of genetic testing for patients with a higher genotype predictor score.¹⁵ In the 6 markers of the Mayo Clinic genetic test prediction score, a reverse curve septal shape might be unfamiliar; however, Binder et al previously reported that reverse-curve HCM was associated with positive genetic testing among the septal morphological subtypes (reverse curve, sigmoid, neutral, and apical subtypes) in HCM.¹¹ We also reported that a reverse curve LV shape was frequently present even in the elderly HCM patients when there were *MYBPC3* mutations.¹⁶

The present study, to the best of our knowledge, is the first study on the use of the Mayo Clinic prediction score in a Japanese HCM population. Phenotypes of Japanese HCM patients differ partially from those in Western countries. For example, apical HCM is known to be more frequent in Japan. We previously demonstrated, by using uniform echocardiographic criteria, that the prevalence of the morphologic apical form of HCM was significantly higher in Japanese patients than in American patients.¹⁷ In our current study, we found that 38 patients (18%) had apical

HCM. In contrast, in the original derivation HCM cohort of the Mayo Clinic, apical HCM was observed in only 7% of the whole cohort.¹⁰ In yet another aspect, age at diagnosis of HCM in our study was 56 ± 16 years and age at diagnosis in the original Mayo Clinic study was 44 ± 19 years. This age distribution of patients in the present study is probably due to the fact that Kochi Prefecture, where our study was performed, is located far from urban areas and is one of the most aged communities in Japan. We think that, at least in Japanese rural regions, many patients with this disease are middle-aged or elderly, despite the fact that HCM is regarded as a genetic disorder. Although there were some differences of phenotypic expression between these 2 cohorts, the Mayo Clinic phenotype-based genetic test prediction score was still useful for providing the pre-test probability of a positive HCM genetic test in Japanese patients. This prediction score consists of easy-to-assess clinical parameters that can help clinicians, the patients themselves, and their families determine whether to pursue genetic testing.

Study Limitations

Our study has some limitations. First, in our study, genetic examination was conducted by using direct sequencing that targeted protein-coding exons of 6 sarcomere genes. In contrast, 9 sarcomere genes were analyzed in the original Mayo Clinic HCM cohort. With regard to the yield of genetic testing, there were similar results for the original cohort and our cohort: genotype-positive rates of 35% in the overall cohort and 53% in patients with a family history of HCM in the Mayo Clinic cohort vs. 26% in the overall cohort and 60% in patients with a family history of HCM in the Kochi cohort, though the criteria for the pathogenicity of detected variants were different. In the future, further research based on data for the whole exome sequence or the whole genome sequence using a next-generation sequencer is needed. Second, in our patient population, there was no difference in maximum LV wall

thickness between genotype-positive and genotype-negative patients, although the original cohort of the Mayo Clinic showed the significant difference between the 2 groups and its cut-off value of 20 mm. It is unclear why our results did not show a significant difference in this regard. Third, our data were from a single center in a rural area; therefore, a further study based on a large and multicenter cohort in Japan is needed.

Conclusions

The Mayo Clinic phenotype-based genetic test prediction score is useful for predicting a positive genetic test result in Japanese HCM patients.

Sources of Funding

This work was supported, in part, by a research grant from the Japan Society for the Promotion of Science to T. Kubo (16K09440).

Disclosures

The authors declare no conflicts of interest. H.K. is a member of *Circulation Journal* Editorial Team.

IRB Information

This study was approved by the Ethics Committee on Medical Research for the Kochi Medical School (reference number ERB-002361).

References

1. Gersh BJ, Maron BJ, Bonow RO, Dearani JA, Fifer MA, Link MS, et al. 2011 ACCF/AHA Guideline for the Diagnosis and Treatment of Hypertrophic Cardiomyopathy: A Report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *Circulation* 2011; **124**: e783–e831.
2. Elliott PM, Anastasakis A, Borger MA, Borggrefe M, Cecchi F, Charron P, et al. 2014 ESC Guidelines on diagnosis and management of hypertrophic cardiomyopathy: The Task Force for the Diagnosis and Management of Hypertrophic Cardiomyopathy of the European Society of Cardiology (ESC). *Eur Heart J* 2014; **35**: 2733–2779.
3. Kitaoka H, Kubo T, Doi YL. Hypertrophic cardiomyopathy: A heterogeneous and lifelong disease in real world. *Circ J* 2020; **84**: 1218–1226.
4. Geisterfer-Lowrance AA, Kass S, Tanigawa G, Vosberg HP, McKenna W, Seidman CE, et al. A molecular basis for familial hypertrophic cardiomyopathy: A beta cardiac myosin heavy chain gene missense mutation. *Cell* 1990; **62**: 999–1006.
5. Kimura A. Molecular etiology and pathogenesis of hereditary cardiomyopathy. *Circ J* 2018; **72**(Suppl A): A38–A48.
6. Olivetto I, Girolami F, Ackerman MJ, Nistri S, Bos JM, Zachara

- E, et al. Myofilament protein gene mutation screening and outcome of patients with hypertrophic cardiomyopathy. *Mayo Clin Proc* 2008; **83**: 630–638.
7. Ingles J, Burns C, Bagnall RD, Lam L, Yeates L, Sarina T, et al. Nonfamilial hypertrophic cardiomyopathy: Prevalence, natural history, and clinical implications. *Circ Cardiovasc Genet* 2017; **10**: e001620.
8. Ho CY, Day SM, Ashley EA, Michels M, Pereira AC, Jacoby D, et al. Genotype and lifetime burden of disease in hypertrophic cardiomyopathy: Insights from the Sarcomeric Human Cardiomyopathy Registry (SHaRe). *Circulation* 2018; **138**: 1387–1398.
9. Nakashima Y, Kubo T, Sugiura K, Ochi Y, Takahashi A, Baba Y, et al. Lifelong clinical impact of the presence of sarcomere gene mutation in Japanese patients with hypertrophic cardiomyopathy. *Circ J* 2020; **84**: 1846–1853.
10. Bos JM, Will ML, Gersh BJ, Kruisselbrink TM, Ommen SR, Ackerman MJ. Characterization of a phenotype-based genetic test prediction score for unrelated patients with hypertrophic cardiomyopathy. *Mayo Clin Proc* 2014; **89**: 727–737.
11. Binder J, Ommen SR, Gersh BJ, Van Driest SL, Tajik AJ, Nishimura RA, et al. Echocardiography-guided genetic testing in hypertrophic cardiomyopathy: Septal morphological features predict the presence of myofilament mutations. *Mayo Clin Proc* 2006; **81**: 459–467.
12. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015; **17**: 405–424.
13. Tsutsui H, Kitaoka H, Isobe M, Ide T, Ueta H, Ono M, et al. JCS 2018 Guideline on Diagnosis and Treatment of Cardiomyopathies (in Japanese). https://www.j-circ.or.jp/cms/wp-content/uploads/2020/02/JCS2018_tsutsui_kitaoka.pdf (accessed May 30, 2020).
14. Otsuka H, Arimura T, Abe T, Kawai H, Aizawa Y, Kubo T, et al. Prevalence and distribution of sarcomeric gene mutations in Japanese patients with familial hypertrophic cardiomyopathy. *Circ J* 2012; **76**: 453–461.
15. Murphy SL, Anderson JH, Kapplinger JD, Kruisselbrink TM, Gersh BJ, Ommen SR, et al. Evaluation of the Mayo Clinic phenotype-based genotype predictor score in patients with clinically diagnosed hypertrophic cardiomyopathy. *J Cardiovasc Transl Res* 2016; **9**: 153–161.
16. Hirota T, Kitaoka H, Kubo T, Okawa M, Furuno T, Doi YL. Morphologic characteristics of hypertrophic cardiomyopathy of the elderly with cardiac myosin-binding protein C gene mutations. *Circ J* 2006; **70**: 875–879.
17. Kitaoka H, Doi Y, Casey SA, Hitomi N, Furuno T, Maron BJ. Comparison of prevalence of apical hypertrophic cardiomyopathy in Japan and the United States. *Am J Cardiol* 2003; **92**: 1183–1186.

Supplementary Files

Please find supplementary file(s);
<http://dx.doi.org/10.1253/circj.CJ-20-0826>