

Genetic anticipation in a special form of hypertrophic cardiomyopathy with sudden cardiac death in a family with 74 members across 5 generations

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Abstract

Hypertrophic cardiomyopathy (HCM) is the most common heritable heart disease. The genetic anticipation of HCM and its associated etiology, sudden cardiac death (SCD), remains unclear. The aim of this study was to investigate the mechanism underlying the genetic anticipation of HCM and associated SCD.

An HCM family including 5 generations and 74 members was studied. Two-dimensional echocardiography was performed to diagnose HCM. The age of onset of HCM was defined as the age at first diagnosis according to hospital records. The information on SCD was confirmed by verification by ≥ 2 family members and a review of hospital records. Whole-genome sequencing was performed on 4 HCM subjects and 1 healthy control in the family. The identified mutations were screened in all available family members and 216 unrelated healthy controls by Sanger sequencing.

The median ages of onset of HCM were 63.5, 38.5, and 18.0 years in members of the second, third, and fourth generations of the family, respectively, and the differences between the generations were significant ($P < 0.001$). The age at SCD also decreased with each subsequent generation ($P < 0.05$). In particular, among the third-generation family members, SCD occurred between 30 and 40 years of age at approximately 8 AM, whereas among the fourth-generation family members, all 5 males who experienced SCD were 16 years of age and died at approximately 8 AM. The sarcomere gene mutations *MYH7-A719H* and *MYOZ2-L169G* were detected in the HCM individuals in this pedigree. Increases in the number of mutations and the frequency of multiple gene mutations were observed in the younger generations. Moreover, a structural variant was present in the HCM phenotype-positive subjects but was absent in the HCM phenotype-negative subjects.

HCM may exhibit genetic anticipation, with a decreased age of onset and increased severity in successive generations. Multiple gene mutations may contribute to genetic anticipation in HCM and thus may be of prognostic value.

Abbreviations: ECG = electrocardiogram, HCM = hypertrophic cardiomyopathy, LV = left ventricular, SCD = sudden cardiac death, SNP = single-nucleotide polymorphism, SV = structural variant, WGS = whole-genome sequencing.

Keywords: genetic anticipation, hypertrophic cardiomyopathy, sudden cardiac death, whole-genome sequencing

Editor: Roman Leischik.

This work was supported by the National Science and Technology Major Project (no. 2012ZX09303-008-001) of the Ministry of Science and Technology of China, the Key Laboratory Construction Project of the Ministry of Health in China, the Graduate Innovation Foundation of the Chinese Academy of Medical Science & Peking Union Medical College, the National Natural Science Foundation of China (81370222), and a research award from the National 1000-Talent Plan of China.

The authors have no conflicts of interest to disclose.

Supplemental Digital Content is available for this article.

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Medicine (2017) 96:11(e6249)

Received: 11 April 2016 / Received in final form: 5 February 2017 / Accepted: 8 February 2017

<http://dx.doi.org/10.1097/MD.0000000000006249>

1. Introduction

Hypertrophic cardiomyopathy (HCM), the most common hereditary cardiac disease, affects 1 in every 500 people in the general population and represents a major cause of sudden cardiac death (SCD) in adolescent athletes.^[1] It exhibits vast genetic and clinical heterogeneity and is typically inherited in an autosomal dominant manner.^[2] More than 1400 variations in ≥ 27 genes encoding proteins in cardiac sarcomeres have been reported to be responsible for (or associated with) HCM over the past 20 years.^[3] Most HCM patients are diagnosed between the ages of 30 and 60, and this disease is relatively infrequent in individuals younger than 25 years of age.^[4] Genetic anticipation is a phenomenon by which the age of onset of an inherited disorder decreases and/or the phenotypic severity increases in successive generations.^[5] The occurrence of this phenomenon has been well established in a number of inherited neurodegenerative disorders and other autosomal dominant genetic diseases and has long been suspected in HCM. Patients with a family history of HCM tend to exhibit symptoms earlier and to have more severe symptoms, such as an increased degree of myocardium hypertrophy or premature

SCD, compared with those without a family history. Czechs et al described the anticipation phenomenon in HCM patients; however, the underlying genetic mechanisms remain unclear.^[6] Gene mutations may occur when specific genetic regions (including noncoding regions) are passed on to subsequent generations. Here, we report the clinical and genetic findings for a multigeneration Chinese family in which genetic anticipation coexists with premature SCD. This is the first study to reveal a genetic basis for the anticipation phenomenon in HCM.

2. Methods

2.1. Study population, pedigree characteristics, and diagnostic criteria for HCM

We identified a large Chinese family with HCM through Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College (Beijing, China). This family included 5 generations and a total of 74 members (41 males and 33 females). The proband was a 47-year-old male (III:15; Fig. 1). We examined all living family

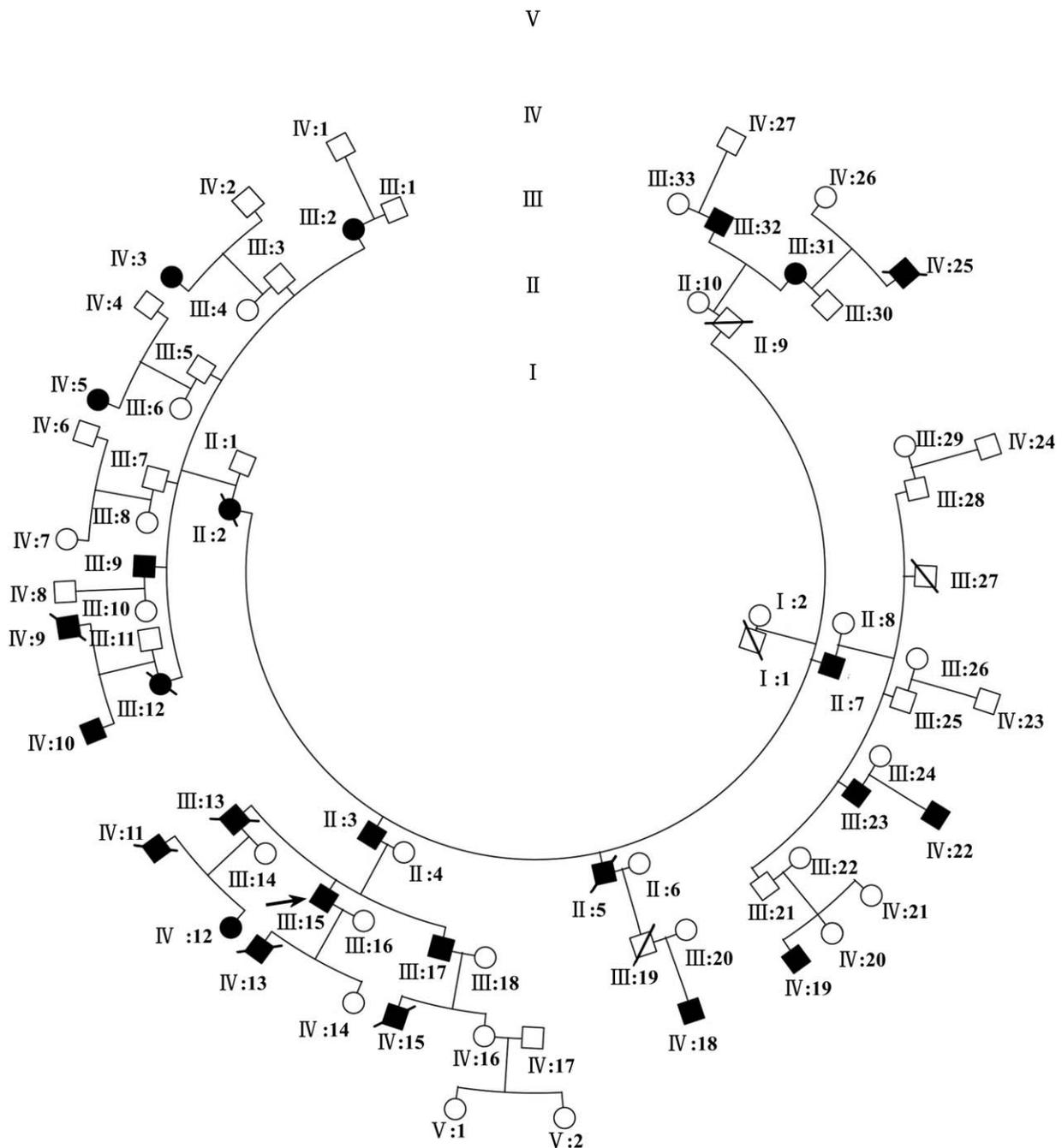


Figure 1. Pedigree of the HCM family. The white symbols represent healthy individuals, the black symbols represent affected individuals, the squares indicate males, and the circles indicate females. The crossed black symbols represent deceased individuals who died of sudden cardiac death. The crossed white symbols indicate deceased individuals who died of other or unknown causes. The individual indicated by the arrow is the proband. The subjects highlighted by asterisks (II:3, III:15, IV:13, IV:14, and IV:22) were examined by whole-genome sequencing. HCM = hypertrophic cardiomyopathy.

members by transthoracic echocardiography. Considering the apparent family history of HCM, the disease was diagnosed based on a left ventricular (LV) wall thickness ≥ 13 mm in adults or a thickness ≥ 2 standard deviations above the predicted mean in children at the end of the diastolic period.^[7] All measurements were performed during an average of 3 cardiac cycles for patients with normal sinus rhythm and a minimum of 5 beats in patients with atrial fibrillation to account for interbeat variability. The LV wall thickness and LV dimensions were measured perpendicular to the long axis of the left ventricle by M-mode imaging and 2-dimensional echocardiography.^[8] Other factors known to cause the observed hypertrophy were not observed in this family. Pulsed-wave, continuous-wave, and color Doppler were used as necessary for accurate definitions of obstruction. SCD was defined as the occurrence of collapse in the absence of or within 1 hour of the onset of symptoms in patients who had previously experienced a relatively stable or uneventful clinical course. The age of onset of HCM was defined as the age at first diagnosis in the hospital setting. The age and time of SCD were confirmed by verification with ≥ 2 family members and by reviewing hospital records.

This study was performed in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants, and all experiments were approved by the Research Ethics Board of Fuwai Hospital.

2.2. Whole-genome sequencing

We selected an immediate family member from each generation (II:3, III:15, and IV:13) to identify genetic alterations. An individual with collateral consanguinity to the proband, IV:22, was also selected to confirm the potential pathogenic mutations. Individual IV:14 had a normal phenotype and was selected as a control for whole-genome sequencing (WGS). Genomic DNA (1.5 μ g) was sheared into ~ 350 -bp fragments using a Covaris S220 sonicator. The concentrations and size distributions of the DNA libraries were determined using an Agilent BioAnalyzer DNA 1000 chip. Paired-end 150-bp reads from the DNA libraries were sequenced using an Illumina HiSeq X system (Illumina, San Diego, CA) according to the manufacturer's instructions. After we performed quality control measures to validate the raw data, the cleaned sequencing reads were mapped to the reference genome (UCSCChg19) to obtain the original mapping results. Picard, GATK, and SamTools were used to remove duplicate reads. In addition, the SamTools mpileup and bcftools were used for variant calling and to identify single-nucleotide polymorphisms (SNPs) and indels. Further, Control-FREEC was used to identify copy number variants, and Crest was used to detect structural variants (SVs).

2.3. Pathogenic variant detection

To search for potential pathogenic mutations, we first removed all noncoding and synonymous variants. Then, rare (frequency $< 0.5\%$) nonsynonymous, loss-of-function, and splice-site variants were selected as candidates. Next, we screened all known cardiovascular disease-associated genes (including an arrhythmia- and cardiomyopathy-associated gene in the Pan Cardiomyopathy Panel) to identify potential pathogenic mutations among the detected variants.^[9,10] Potential pathogenic mutations were additionally required to be absent from publicly available databases, including dbSNP, the 1000 Genomes Project, and the National Heart, Lung, and Blood

Institute Grand Opportunity Exome Sequencing Project (ESP 6500).^[11] For the SNPs, we also used SIFT and PolyPhen-2 software programs to predict their protein structures and functional alterations. The detected potential pathogenic mutations were validated by Sanger sequencing with an ABI3130XL Genetic Analyzer (Applied Biosystems, Foster City, CA) in the corresponding individuals to avoid false-positive results in WGS. The novel mutations were confirmed in 216 unrelated healthy individuals.

2.4. Cosegregation analysis of the family

Shared variants that were detected in the affected individuals but not in the healthy individuals in this family were selected and further validated by Sanger sequencing in all family members. These mutations were considered pathogenic/nonpathogenic based on the preponderance of evidence obtained from cosegregation analysis (whether the identified mutations cosegregated with the HCM phenotype). The novel identified mutations were also confirmed in 216 unrelated healthy individuals.

2.5. Statistical analysis

Continuous variables are expressed as the median and interquartile range. Nonparametric tests were used to compare the age of onset of HCM. Parent-child generations with HCM were compared using the Wilcoxon rank-sum test. The age of onset of HCM was compared across generations using the Kruskal-Wallis test. For subjects with different numbers of mutations, the age of onset of HCM, echocardiographic parameters, and predicted probability of SCD at 5 years were compared using the Kruskal-Wallis test, and the rate of cardiovascular events was compared using Fisher exact test. Analyses were conducted using SPSS 16.0 software (SPSS Inc, Chicago, IL). A P value < 0.05 was considered to indicate statistical significance.

3. Results

3.1. Clinical investigation of the pedigree

The proband, a 47-year-old male (III:15; Fig. 1), was referred for evaluation due to obvious chest tightness and was diagnosed with HCM at 37 years of age. His brother (III:13; Fig. 1) and cousin (III:12; Fig. 1) died of SCD at 35 and 30 years of age, respectively, both at approximately 8 AM. The proband's father (II:3; Fig. 1) was diagnosed with HCM when he was 68 years old. His aunt and uncle (II:2 and II:5, respectively; Fig. 1) died suddenly at 49 and 59 years of age, respectively. The proband's 4 nephews experienced SCD at 16 years of age, all at approximately 8 AM. During the follow-up period, the proband's 16-year-old son collapsed and became unconscious while studying in class at approximately 8 AM. Electrocardiogram (ECG) revealed ventricular fibrillation, and the boy died in the emergency room.

In addition to the 8 family members who died of SCD due to HCM, 17 living family members were diagnosed with HCM by echocardiography or cardiac magnetic resonance (CMR) examination. Thus, a total of 25 persons were affected with HCM in this family. The incidence in males (56%, 14/25) was similar to that in females (44%, 11/25) at the most recent follow-up (July 2015). The basic characteristics of the pedigree are shown in Table 1.

Table 1**The basic characteristics of the pedigree.**

Generation	Number of subjects	Number with HCM	Number with SCD	Number with HCM and SCD	Age at onset	Age of SCD
II	10	4	4	2	63.5 (53.5, 71.5)	53.5 (48.0, 59.0)
III	33	9	4	2	38.5 (32.5, 42.5)	32.5 (30.0, 35.0)
IV	27	12	5	5	18.0 (14.0, 19.0)	16.0 (16.0, 16.0)
V	2	0	0	0		
Total	72	25	13	9	28.0 (18.0, 44.0)	16.0 (16.0, 35.0)

Age is presented as medians and quantiles. HCM = hypertrophic cardiomyopathy, SCD = sudden cardiac death.

Table 2**Summary of single-nucleotide polymorphism mutations in the pedigree.**

Gene	Chromosome	Full gene name	Exon	DNA change	Amino acid change	Codon change	Published/de novo	Published before/de novo
<i>MYH7</i>	14	Myosin, heavy chain 7, cardiac muscle, beta	3	2155C>T	Arg719Trp	Cgg/Tgg	Published	Published before
<i>MYOZ2</i>	4	Myozenin 2	4	505A>C	Lys169Gln	Aaa/Caa	De novo	De novo
<i>DNAJC19</i>	3	DnaJ (Hsp40) homolog, subfamily C, member 19	3	59G>A	Arg20His	cGt/cAt	De novo	De novo
<i>CTNNA3</i>	10	Catenin (cadherin-associated protein), alpha 3	2	2441T>A	Leu814*	tTa/tAa	De novo	De novo
<i>NEBL</i>	10	Nebulette	2	1286C>T	Ser429Leu	tCa/tTa	De novo	De novo
<i>TTN</i>	2	Titin	2	90407T>C	Ile30136Thr	aTc/aCc	De novo	De novo

3.2. Genetic anticipation

In this family, the median age of onset of HCM was 28.0 years (range, 8–75 years). The median ages of onset were 63.5 years among the second-generation family members, 38.5 years among the third-generation members, and 18.0 years among the fourth-generation members. The age of onset of HCM differed significantly between each pair of generations (Wilcoxon rank-sum test; Fig. 2) as well as among all 5 generations ($P < 0.0001$, Kruskal–Wallis test; Fig. 2). SCD occurred in the second-generation family members at approximately 50 to 60 years of age and in the third-generation family members at approximately 30 years of age at 8 AM. Surprisingly, among the fourth-generation family members, SCD occurred at 8 AM in individuals who were 16 years of age ($P = 0.0198$, Kruskal–Wallis test; Fig. 2). In summary, the age of onset of HCM among the affected

family members decreased and the severity of the clinical phenotype increased with each successive generation, with higher rates of heart failure, syncope, and SCD observed in the younger generations.

3.3. WGS results

WGS analysis revealed the presence of heterozygous mutations in 2 distinct sarcomere genes in the 4 affected individuals: 2 missense mutations were detected in *MYH7* (c.2155C>T in exon 3, A719H) and *MYOZ2* (c.505A>C in exon 4, L169G). Furthermore, a heterozygous missense mutation in *DNAJC19* (c.59G>A in exon 3, A20H) and a heterozygous nonsense mutation in *CTNNA3* (c.2441T>A in exon 2, Leu814*) were detected in the proband. In addition to these 4 mutations, heterozygous missense mutations in *TTN* (c.90407T>C in exon 2, I30136T) and *NEBL* (c.1286C>T in exon 2, S429L) were identified in the proband's son (Table 2). Only *MYH7-A719H* has been previously reported in association with HCM.^[12,13] The proband's 20-year-old daughter (IV:14; Fig. 1) carried only the *MYH7-A719H* mutation and was clinically unaffected, with normal ECG and echocardiogram findings. The echocardiogram results and corresponding mutations observed in the 3 immediate generations are shown in Fig. 3.

In addition to SNPs, a large deletion SV (~400 bp) was identified in the intragenic region between the *MYH7* and *NGDN* genes in 4 of the subjects with the HCM phenotype by WGS. However, this SV was not detected in the phenotype-negative individual IV:14.

3.4. Genotype–phenotype correlations

To determine the genotype–phenotype relationship as well as the pathogenesis associated with each of the new mutations identified in this family, the subjects' clinical features, ECG examination results, and prognoses were further analyzed. Within this family (Table 3), 12 individuals carried *MYH7-A719H*, and 16 carried *MYOZ2-L169G*. Among the 12 subjects who were clinically

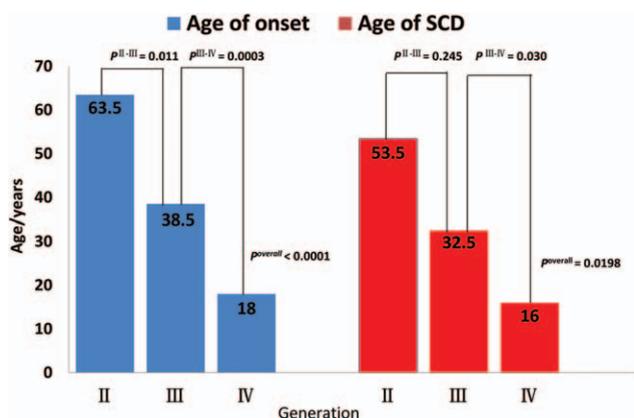


Figure 2. The median age of onset of HCM and median age of SCD across generations. The ages of onset of HCM and SCD were clearly lower in the younger generations. HCM = hypertrophic cardiomyopathy, P^{II-III} = P value between generations II and III (Wilcoxon rank-sum test), P^{III-IV} = P value between generations III and IV (Wilcoxon rank-sum test), $P^{overall}$ = P value among all generations (Kruskal–Wallis test), SCD = sudden cardiac death.

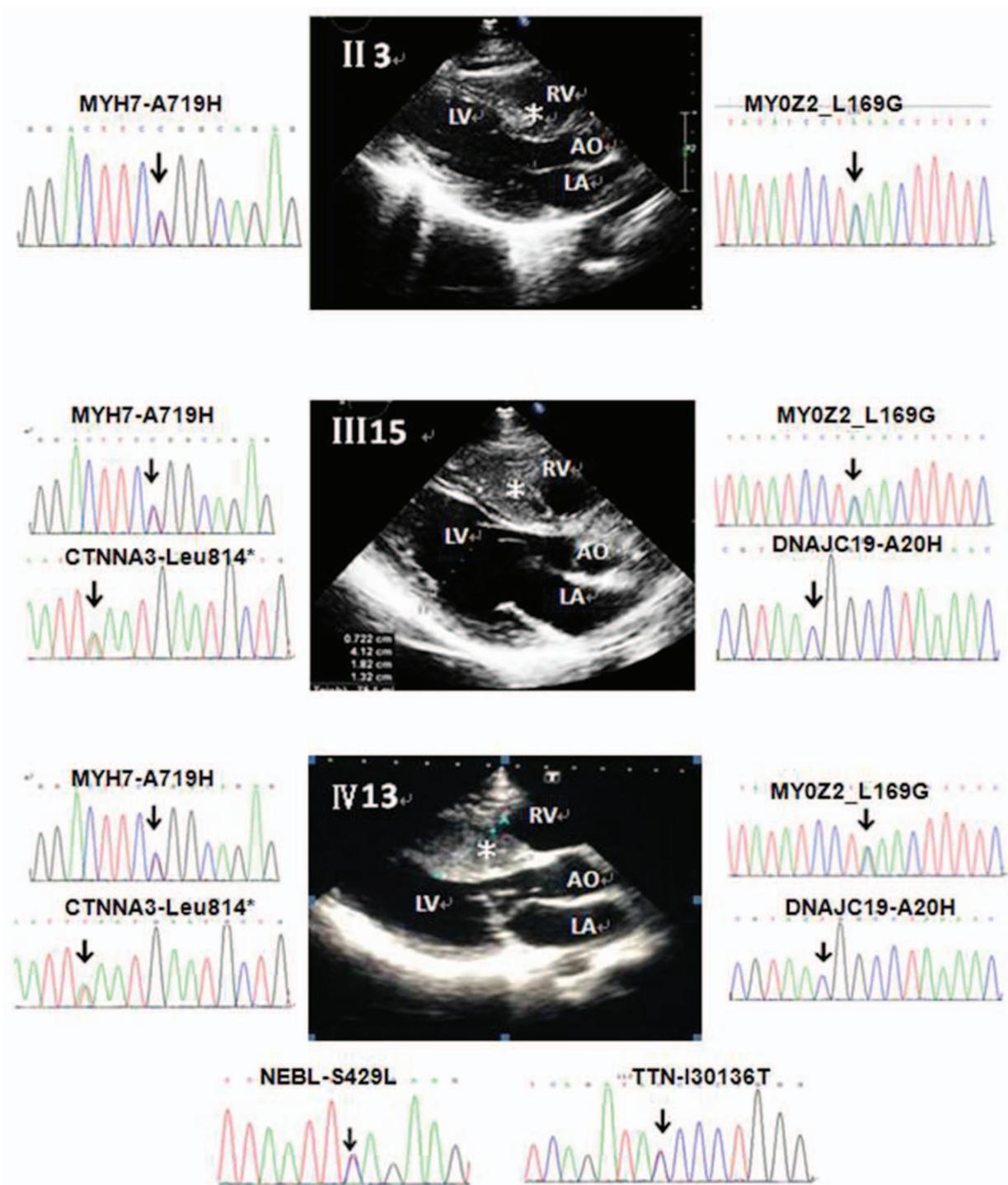


Figure 3. Electropherograms of the identified mutations in the affected HCM patients. Two-dimensional echocardiography in the parasternal left ventricular long-axis view showing increasing hypertrophy in the interventricular septum of the 3 affected individuals. The asterisk indicates the maximum hypertrophy at this site in each individual. Around the echocardiography, the vertical rectangles and the black arrows indicate the sites of the identified mutations. AO = aorta, HCM = hypertrophic cardiomyopathy, LA = left atrium, LV = left ventricle, RV = right ventricle.

diagnosed with HCM, 7 carried both *MYH7-A719H* and *MYOZ2-L169G*, 4 had only *MYH7-A719H*, and 1 did not possess either mutation. Interestingly, sequencing of the *MYH7* gene in all family members revealed that those who carried only the *MYH7* mutation had mild LV hypertrophy or normal heart function and morphology, and all experienced only chest

discomfort or no symptoms at all after activities. Twenty-four-hour Holter monitoring revealed very mild premature ventricular or atrial contractions, and ECG indicated ST-T abnormalities. By contrast, the 7 patients with both *MYH7-A719H* and *MYOZ2-L169G* had more serious symptoms, commonly complaining of palpitation and dyspnea. During the follow-up period, 1 of these

Table 3**Relationships between genotypes and phenotypes in the study pedigree.**

Genotype	Sex	Onset age	Symptoms	HCM	LAD, mm	LVEDD, mm	IVST maximum, mm	LVPWT maximum, mm	Probability SCD at 5 y, %	Prognosis
<i>MYH7+MYOZ2</i>										
II:3	M	68	2, 5	+	31	39	18	9	1.5	Progressive HF
II:7	M	75	2	+	30	43	13	11	0.9	Progressive HF
III:15	M	37	2	+	38	52	20	8	3.2	Stable
III:23	M	40	2	+	25	42	16	10	1.8	MI
III:9	M	48	2	+	27	41	17	11	1.7	Stable
IV:13	M	8	0	+	36	36	16	10	8.5	SCD
IV:22	M	23	1, 2, 3	+	37	43	20	14	4.2	Stable
<i>MYH7</i>										
III:2	F	28	1, 2, 3, 5	+	35	41	22	12	3.8	Stable
III:17	M	44	2	+	34	48	16	11	2.1	Stable
III:32	M	41	2, 3	+	25	45	16	17	1.8	Stable
IV:12	F	17	1, 2	+	26	35	26	9	4.0	Stable
IV:14	F	21	0	–	26	42	10	10	1.6	Stable
<i>MYOZ2</i>										
III:28	M	46	0	–	24	25	10	11	1.0	Stable
III:3	M	36	0	–	29	47	10	10	1.3	Stable
III:5	M	36	0	–	25	40	11	11	1.3	Stable
III:7	M	44	0	–	30	45	11	10	1.3	Stable
IV:20	F	16	0	–	23	41	11	11	1.8	Stable
IV:23	M	14	0	–	26	38	9	8	1.6	Stable
IV:2	F	11	0	–	23	39	10	9	1.8	Stable
IV:4	M	10	0	–	18	33	8	7	1.3	Stable
IV:6	M	17	0	–	25	38	8	9	1.4	Stable

Symptoms: 1 = chest pain, 2 = chest tightness, 3 = palpitations, 4 = dizziness, 5 = dyspnea. F = female, HCM = hypertrophic cardiomyopathy, HF = heart failure, IVST = interventricular septum thickness, LAD = left atrial diameter, LVEDD = left ventricular end-diastolic diameter, LVPWT = left ventricular posterior wall thickness, M = male, MI = myocardial infarction, SCD = sudden cardiac death.

subjects experienced SCD, and another suffered from myocardial infarction without obvious hyperlipidemia. Ten of the subjects who carried only the *MYOZ2* mutation showed no LV hypertrophy, abnormal ECG or echocardiographic findings, or discomfort. Compared with subjects with only *MYH7-A719H* or *MYOZ2-L169G* mutation, patients with both the *MYH7-A719H* and *MYOZ2-L169G* mutations had the largest median left atrial diameter (Kruskal–Wallis test, $P=0.015$) and the highest frequency of cardiovascular events (Fisher exact test, $P=0.007$). Using the HCM Risk-SCD formula, we found that individual IV:13 possessed the highest probability of experiencing SCD among all family members. Family members with both *MYH7-A719H* and *MYOZ2-L169G* mutations also showed the greatest median probability of SCD at 5 years (Kruskal–Wallis test, $P=0.033$). The potential risks of SCD for all individuals in the family were calculated and are shown in Table 3.

4. Discussion

Genetic anticipation is a characteristic of autosomal dominant diseases. It has been previously described in HCM, but its genetic basis has not been reported. It has been suggested that the age of onset alone may not be a valid criterion for detecting anticipation in patients with complex diseases. For example, medical care is more accessible to younger generations, and the sensitivity of diagnostic tools has improved over time.^[14] Thus, aggravated HCM, which can be distinguished based on the anticipated age of SCD, could serve as fairly strong evidence of anticipation. More severe symptoms and the acceleration of the disease process led to premature death in the younger generation of family members. In reentrant ventricular tachyarrhythmia, the mechanism underlying SCD is probably associated with an electrically unstable and unpredictable myocardial substrate.^[1] Notably, individual IV:13

experienced ventricular tachycardia attack in the emergency room during SCD (see the figure in the Supplemental Content, which shows the ECG findings, <http://links.lww.com/MD/B598>). Histopathological markers of disorganized myocardial architecture and replacement scarring may have caused these malignant arrhythmias^[1] (as indicated by obvious regional transmural late gadolinium enhancement on CMR imaging in IV:13).

WGS may revolutionize medical diagnostics by enabling the rapid identification of alleles that cause disease. However, even in cases with a simple pattern of inheritance and an unambiguous diagnosis, the relationship between the disease phenotype and the corresponding genetic alterations can be complicated.^[15] Pedigree cosegregation analyses have sufficient power to identify disease-causing mutations and determine their pathogenicities in patients with HCM.^[9] The construction of a 3- or 4-generation family pedigree is useful for confirming the genetic origin of a disease and for identifying other family members who are at risk of developing it.^[16] In our study, we first selected a rare mutation by filtering our data through comprehensive databases. We then used new software and techniques (e.g., SIFT and PolyPhen-2) to predict protein structural configurations and functional alterations. By performing these steps, we increased the chance that the identified mutations were causative or, at the very least, modulators of the HCM phenotype.^[17] The mutations were cosegregated with the HCM phenotype within the study family, and finally, we determined whether each mutation was likely pathogenic or nonpathogenic based on the cosegregation results.

4.1. Potential mechanisms underlying genetic anticipation in the study HCM family

Genetic anticipation can result from events such as trinucleotide repeat expansion, mitochondrial alterations, and changes in gene

dosage. The generational expansion of trinucleotide repeats that occurs during meiosis and gametogenesis represents a molecular mechanism explaining the earlier onsets and worse prognoses of affected individuals observed in successive generations of families with several neurodegenerative disorders, including Huntington chorea and spinocerebellar ataxia.^[18] Other potentially implicated mechanisms include mitochondrial heteroplasmy or the aberrant expression of modifier genes that segregate independently of the disease gene.^[19] Alterations in gene dosages resulting from dominant mutations can also explain anticipation. Multiple gene mutations may be associated with more severe symptoms and a worse prognosis, such as markedly increased risks of rapid end-stage progression and ventricular arrhythmia in HCM patients. Moreover, heterozygotes exhibit incomplete penetrance and may develop late-onset disease, whereas homozygotes and compound heterozygotes exhibit accelerated disease processes, leading to early onset or premature sudden death.^[20] In the present study, we conducted WGS analysis to examine genomic variations in 5 members of 3 successive generations of an HCM family who exhibited clear signs of anticipation and a specific phenotype, SCD. The WGS results showed that the number of gene mutations was increased in the younger generation; therefore, the anticipation that occurred in our study might be explained by the gene dosage theory. HCM patients with double or triple gene mutations exhibit extensive cardiac remodeling, including restrictive physiology, atrial dilation, and systolic dysfunction and require cardiac transplantation.^[21,22] Multiple sarcomere mutations have been associated with the risk of sudden death, even in the absence of conventional risk factors.^[23] In the family studied in this report, an increased number of mutations was associated with a younger age of onset and aggravated disease severity. Moreover, although both sarcomere gene mutations (*MYH7* and *MYOZ2*) were present in autosomal genes, double mutations in these genes were detected only in males. This finding might have been a coincidence, but it could help to explain why males exhibited more severe symptoms and worse prognoses. The presence of multiple abnormal myofilament proteins resulting from complex combinations of gene mutations may be associated with more profound disruption of sarcomere mechanics and myocardial energetics, in addition to cardiomyocyte dysfunction. This may potentially explain the effects of multiple mutations in these patients.^[24]

4.2. Importance of the *MYOZ2-L169G* mutation

MYOZ2 encodes a protein belonging to the myozenin family, which includes Z-disk proteins that are exclusively present in striated muscles that can cause HCM by activating the calcineurin pathway^[25] or that function independently of calcineurin activity in the heart.^[26] In our study, all subjects who carried the *MYOZ2-L169G* mutation were heterozygotes. Ten of these subjects carried only this mutation, and their clinical manifestations and ECG findings were normal, supporting the notion that *MYOZ2-L169G* had few pathogenic effects in this family. However, among the 5 carriers of *MYH7-A719H* (determined using WGS), the phenotype-negative daughter (IV:14) did not carry the *MYOZ2-L169G* mutation, whereas the phenotype-positive grandfather (II:3), father (III:15), brother (IV:13), and cousin (IV:22) carried it. Moreover, the family members who carried both the *MYH7* and *MYOZ2* mutations had more severe symptoms, including SCD, than those with only the *MYH7* mutation. It can be inferred that *MYOZ2* may play a

modifying role in HCM by affecting the penetrance or degree of performance of the *MYH7* gene. However, the cumulative effects of the mutations on the disease phenotype may represent a new feature of HCM. Therefore, *MYOZ2* could be an additive gene, whereas *MYH7* could be a major gene in HCM.

4.3. Potential effects of SVs on HCM

The effects of abnormal SVs on HCM have not been reported. A large deletion (~400 bp) was detected in 4 of the phenotype-positive family members in this study (II:3, III:15, IV:13, and IV:22), but it was not identified in the phenotype-negative individual IV:14. More interestingly, this SV was associated with the presence of the *MYOZ2-L169G* mutation, which might be evidence of a potential interaction between different mutations. Unbalanced gene rearrangements, such as insertions, deletions, and tandem duplications, may lead to changes in gene dosage, which can affect the carrier phenotype.^[27] An SV in a noncoding region may substantially alter the position of a regulatory element that controls gene expression or affects its regulation (i.e., position effects), thereby modulating the expression of the gene. An SV can also affect gene expression by changing local chromatin structure.^[28] In addition, although some SVs do not result in phenotypic alteration, they can impact disease susceptibility in affected individuals. The SV detected in this family might have affected the phenotypic severity. Therefore, SVs and other types of mutations, such as indels and copy number variations, should be considered in HCM.

4.4. Possible explanation for the heterogeneity observed in HCM

The *CTNNA3* gene mutation can cause arrhythmogenic right ventricular cardiomyopathy, which is characterized by arrhythmia and juvenile sudden death.^[29] The ECG findings for the patients who experienced SCD in this study showed clear signs of ventricular fibrillation attack, which might be the result of the expression of an arrhythmogenic gene mutation. Using high-throughput sequencing, Lopes et al found that approximately 43% of HCM patients carried variants in genes associated with arrhythmogenic right ventricular cardiomyopathy and ion channel diseases, and these variants may explain why some patients with HCM suffer from symptoms ranging from malignant arrhythmia to SCD.^[17] *NEBL* gene mutation has been associated with endocardial fibroelastosis and dilated cardiomyopathy.^[30] The *DNAJC19* gene has also been associated with dilated cardiomyopathy.^[31] Although the significance of the mutations in the 2 genes identified in the current study were more difficult to assess, the variants observed in this pedigree occurred at a frequency of <0.5% in the 1000 Genomes Project. These findings suggest that at the very least, these mutations partly modulated the patients' phenotypes.^[17] The presence and effects of these mutations might explain the earlier and more severe symptoms that were observed and might even contribute to the heterogeneity observed in HCM.

4.5. Potential explanation of the SCD phenotype

The HCM Risk-SCD model has improved risk stratification in HCM for the primary prevention of SCD. The results obtained using this model are in agreement with the observed SCD risks in this family. Individual IV:13 exhibited the highest 5-year risk of developing SCD, and he experienced SCD at 16 years of age.

According to the recommendations of this risk model,^[16] individuals IV:12 and IV:22 should consider cardioverter defibrillator implantation. The increased sympathetic activity that occurs in the morning or during sports may cause hyperfunctionality of the circulatory system, which can include coronary artery constriction and tachycardia, and these symptoms may lead to myocardial ischemia and malignant arrhythmia. This phenomenon may explain the finding that all cases of SCD in this study occurred at approximately 8 AM. However, it is difficult to explain why SCD occurred in the third-generation family members while they were in their 30s, whereas among the fourth-generation family members, SCD occurred only in 16-year-old males. It remains unknown whether these findings were an incredible coincidence or were triggered by changes such as altered hormone levels,^[32] similar degrees of myocardial fibrosis,^[33] the early activation of cellular and molecular responses to or the expression of some mutations,^[34] or the excitation of the sympathetic nervous system. Thus, the mechanism potentially underlying SCD initiation is unknown and requires further study.

Moreover, in young subjects with SCD, it is not any more common to find HCM than a structurally normal heart at the time of death, and confirmation of SCD from arrhythmogenic causes such as channelopathies (long-QT syndrome, short-QT syndrome, etc.) may be required.^[35,36] It is possible that an individual's profile of common cardiac channel polymorphisms might mediate pathogenic susceptibility to arrhythmia in the setting of HCM.^[35,37] Further examination and gene sequencing beyond HCM would be useful in familial heart disease with SCD.

Finally, HCM is still the most important cause of SCD in competitive athletes^[38,39]; thus, cardiovascular screening is important for young athletes and relatives from HCM families to prevent the risk of HCM-associated SCD.^[38] Echocardiography is the most commonly used noninvasive technique in HCM. A comprehensive approach is recommended for the assessment of LV diastolic function and filling pressures in patients with HCM, including speckle tracking, E/e' ratio, LA volume index, and pulmonary vein atrial reversal velocity,^[40,41] to potentially predict adverse outcomes, including death, cardiac arrest, and ventricular tachycardia. Moreover, new parameters such as ultrasound deformation imaging and 2-dimensional strain could provide more sensitive detection of regional myocardial motion and deformation^[42] and quantification of global and regional systolic dysfunction and pathological LV hypertrophy. CMR is a more accurate method for diagnosing HCM due to its unrivaled capability to assess LV hypertrophy and LV fibrosis. A recent study indicated that combined CMR high-resolution quantitative assessment of myocardial perfusion and LV fibrosis is feasible in patients with HCM, in addition to late gadolinium enhancement.^[43] This method could provide more accurate information on the ischemic burden, which may have potential significance in the outcome of HCM.^[44] Other parameters that are considered important markers for cardiovascular prognosis and therapeutic responses, such as LV mass or LV hypertrophy, should also be measured by CMR in HCM.^[43,45]

4.6. Limitations

Some limitations of this study should be considered. First, comprehensive LV parameters were not determined by echocardiography in the clinic in this work, and CMR was not performed in all family members because of financial limitations. Second, as we lacked a simple method, the SVs detected by WGS were not

verified using another technique. Third, we filtered the regions containing noncoding and synonymous variants, which may have resulted in the loss of additional useful genetic information. Therefore, further studies of mutations in other gene regions are needed. Fourth, this study is based on the pedigree of 1 family, and more pedigrees of families with HCM are needed to validate these results. Moreover, this study was confined to the examination of cosegregation in the pedigree, and no functional experiments were performed.

5. Conclusions

Genetic anticipation was observed in the HCM family evaluated in this study. Multiple gene mutations were determined to be the most likely causes of genetic anticipation in the HCM patients in this family. These mutations affected the phenotypes and prognoses of these patients. The *MYOZ2-L169G* mutation alone was not found to be pathogenic, but it did appear to play a modifying role in HCM.

Acknowledgment

We thank Dr Zunsong Hu for assisting with the statistical analyses.

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