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Original article

Detection of mutations in symptomatic patients with hypertrophic cardiomyopathy in Taiwan

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ABSTRACT

Background: Hypertrophic cardiomyopathy (HCM) is a common genetic cardiac disorder associated with sudden death, heart failure, and stroke. The aim of the present study was to evaluate the prevalence and types of mutations in symptomatic patients with HCM in Taiwan.
Methods: Thirty-eight HCM index patients (mean age 60 ± 16 years) underwent systematic mutation screening of eight sarcomeric genes: β-myosin heavy chain (*MYH7*), myosin-binding protein C (*MYBPC3*), troponin T (*TNNT2*), troponin I (*TNNI3*), myosin ventricular regulatory light chain 2 (*MYL2*), myosin ventricular essential light chain 1 (*MYL3*), α-tropomyosin (*TPM1*), and cardiac α-actin (*ACTC*), using direct DNA sequencing. In silico programs predicted damaging amino acids. In the positive families, genotype–phenotype correlation studies were done.
Results: Overall, 13 mutations were identified in 13 index patients (34.2%). The three most frequently mutated genes were *MYH7*, *MYBPC3*, and *TNNT2*. One patient carried double mutations. Five mutations (*MYH7* R147S; *MYBPC3* R597Q; *MYBPC3* W1007R; *TNNI3* E124Q; *MYL3* R63C) were novel; all were missense mutations. Analysis using in silico tools showed near consensus to classify these five novel mutations as pathological. Family pedigree analysis showed the presence of cosegregation in at least two affected members in each proband family, but incomplete penetrance in young family members with a positive genotype.
Conclusions: We identified 13 HCM pedigrees, including 5 carrying novel mutations and 1 with a double mutation. The three most commonly mutated genes were *MYH7*, *MYBPC3*, and *TNNT2*. These results, together with genetic counseling, could lead to earlier diagnosis and better management of family members at risk of HCM.

Introduction

Hypertrophic cardiomyopathy (HCM), clinically defined as thickening of the myocardial wall in the absence of any other cause of left ventricular (LV) hypertrophy, is often inherited genetically, and affects 1:500 individuals [1,2]. The clinical and pathological manifestations are diverse and they range from asymptomatic clinical courses to severe heart failure and sudden cardiac death (SCD). It is caused by 11 or more genes encoding proteins of the cardiac sarcomere [3]. From patients who have been genotyped successfully, around 70% have been found to have mutations in the gene encoding β-myosin heavy chain (*MYH7*) or myosin-binding protein C (*MYBPC3*). Troponin T (*TNNT2*) and several other genes account for 5% or less of cases. Existing data have been largely obtained for Caucasian samples. However, no data have been derived from a systematic screening of HCM from the Taiwanese, who comprise the major population group in Taiwan and are the descendants of early settlers from the southeast coast of China during the past 400 years or more [4,5]. Although there have been a few systematic surveys of gene-proven HCM from Chinese [6,7], the relationships need to be further investigated. Therefore, we investigated the prevalence and type of mutations among unrelated Taiwanese with symptomatic HCM. In all patients, a systematic screening for mutations was performed in eight genes that code for the components of the

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sarcomere: *MYH7*, *MYBPC3*, *TNNT2*, troponin I (*TNNI3*), myosin ventricular regulatory light chain 2 (*MYL2*), myosin ventricular essential light chain 1 (*MYL3*), α-tropomyosin (*TPM1*), and cardiac α actin (*ACTC*).

Materials and methods

Subjects

Unrelated symptomatic adult patients were recruited from two tertiary referral centers (Taipei and Kaohsiung Veterans General Hospitals, Taiwan). Informed consent was obtained from all the patients according to institutional guidelines. The condition was diagnosed following the American College of Cardiology/European Society of Cardiology (ACC/ESC) criteria, using inclusion criteria of a left ventricular wall thickness ≥13 mm on echocardiography when no other cause explained the hypertrophy [8]. The 12-lead electrocardiograms obtained at or near the time of initial HCM diagnosis were assessed. LV hypertrophy by electrocardiographic criteria was adopted by the Sokolow–Lyon criteria: the sum of S1 wave in V1 and R wave in V5 (or V6) >38 mm [9]. Strain pattern was characterized by tall lateral precordial voltages in association with ST–T abnormalities in leads V5 and V6. Prognosis in families was assessed at the time of genotyping and was based on family history. A major cardiac event was defined as sudden death, heart failure death, stroke death, or resuscitated death related to HCM, each occurring before 60 years of age. Probands with any relative diagnosed with HCM were considered familial cases, and patients with proven HCM but without familial history or affected relatives were considered sporadic cases.

Genetic study

Genomic DNA was isolated from the leukocytes of the peripheral blood of the patients. Polymerase chain reaction was used to amplify the exons and flanking intronic bases of the eight genes, including *MYH7* (40 exons), *MYBPC3* (35 exons), *TNNT2* (17 exons), *TNNI3* (8 exons), *MYL2* (7 exons), and *MYL3* (6 exons). When no mutation was found, analysis of the *TPM1* (nine exons) and *ACTC* (six exons) genes was performed. The primers used for the polymerase chain reaction were designed using reference sequences deposited in the GenBank database. Information on the primers and amplification conditions can be obtained from the authors at the correspondence address. Standard DNA sequencing reactions were performed using the fluorescence-labeled dideoxy chain termination method with the Big Dye Terminator ABI Prism Kit and the ABI PRISM™ 3700 DNA Analyzer (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions.

Confirmation of mutation and family genotyping

A variant was considered a mutation in accordance with the following criteria: (1) presence in tested affected members of the family of a proband; (2) absence from 200 unrelated chromosomes of the control subjects; (3) absence from a public database of polymorphisms, the dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/); (4) conservation of the mutated residue among species and isoforms; and/or (5) the gene has been reported as an HCM-causing mutation in the literature. Moreover, the variants were revised to assess their pathogenicity using in silico tools. The topological placement of the mutations was localized using the SwissProt database (http://ca.expasy.org/uniprot/) and the bibliography previously described [10]. The UniProt database provides generally accepted residue ranges corresponding with each domain region and specialized subregion. To predict the pathogenicity of the damaging amino acid substitution, four online tools, SIFT (http://sift.jcvi.org/www/SIFT_seq_submit2.html), Pmut (http://mmb.pcb.ub.es/PMut/), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), and SNAP (http://www.rostlab.org/services/snap/), were used. A missense mutation was assumed to be possibly disease-causing if at least two independent programs indicated a damaging effect. To predict the altered reading frame in the nonsense mutation, the online software Open Reading Frame (ORF) Finder from the NCBI (http://www.ncbi.nlm.nih.gov/projects/gorf/) was used. Family members of the probands with the identified mutations were invited to participate in this investigation, regardless of whether they had symptoms of the disease.

Statistical analysis

The data for continuous variables have been expressed as mean value with ranges and compared using the non-parametric Kruskal–Wallis *H* test. The data for categorical variables have been expressed as numbers or percentages and compared using Fisher's exact test. Data were collected and analyzed using SPSS version 14.0 (SPSS Inc., Chicago, IL, USA). A *p*-value of less than 0.05 was considered statistically significant.

Results

Clinical characteristics and genetic results

The clinical characteristics of all patients are summarized in Table 1. There were no significant differences in sex, symptom severity, LV wall thickness, and presence of LV outflow tract (LVOT) obstruction for the genotype-positive patients and those without mutations, but genotype-positive patients had an earlier age of onset at diagnosis and higher incidence of family history of SCD. The enrolled patients with LVOT >50 mmHg and refractory medications (44.7%) underwent alcohol septal ablation later. Overall, 13 mutations in the selected genes were identified in 13 patients, leading to a genetic diagnosis in 34.2% of the index patients (Table 2). Five mutations were novel.

The genes most frequently involved in the genotype-positive patients were *MYH7* and *MYBPC3*, which were mutated in 46.2% and 30.8% patients, respectively. The other mutated genes (*TNNT2*, *TNNI3*, and *MYL3*) were involved in 30.8% of cases. The distribution of the different mutation types was 92.3% missense ($n = 12$), and 7.7% deletions ($n = 1$). One patient carried double mutations, with a mutation in *MYH7* R858C and another in *TNNT2* R286H. Table 3 shows the echocardiographic and electrocardiographic findings at age of diagnosis in index patients carrying mutations.

To determine the pathogenicity of these mutations, we performed an in silico study and surveyed the family pedigree. Table 4 shows a suite of different tools. The PolyPhen structure-based method predicted that all changes were damaging. In contrast, *MYH7* R663H, E1902Q were identified as a neutral variant according to SNAP; *MYH7* E1902Q, *MYBPC3* Q998E and *TNNI3* E124Q were identified as a neutral variant according to Pmut. Five mutations were novel: one mutation in *MYH7*, two in *MYBPC3*, one in *TNNI3*, and one in *MYL3*. All are missense mutations. Analysis using SIFT, PolyPhen, SNAP, and Pmut showed near consensus for classifying the five novel mutations as pathological.

Pedigree and mutations analysis of these five novel and the double families

Fig. 1 shows the pedigrees of all the families with novel mutations and the double mutation. A person with Proband *MYH7* R147S experienced chest pain at the age of 48 years. Echocardiography showed asymmetric septal hypertrophy (ASH) with mid LV

Table 1
Clinical characteristics of hypertrophic cardiomyopathy patients cohort.

	All patients $n=38$	Genotype positive $n=13$	Genotype negative $n=25$	p-value
Male/female	19/19	9/4	10/15	0.087
Age at diagnosis (years)				
Mean	60.2 ± 16.0	47.1 ± 14.0	67.2 ± 12.2	0.000
Ranges	18–93	18–72	39–93	
Body mass index (kg/m^2)	23.8 ± 2.7	23.0 ± 2.0	24.4 ± 3.0	0.166
Syncope	10 (26.3%)	5 (38.5%)	5 (20.0%)	0.220
Cardiac symptoms (NYHA class I–II)	16 (42.1%)	5 (38.5%)	11 (44.0%)	0.743
Cardiac symptoms (NYHA class III–V)	22 (57.9%)	8 (61.5%)	14 (56.0%)	
Family history of SCD	7 (18.4%)	5 (38.5%)	2 (8.0%)	0.034
LV septal wall thickness (mm)	19.1 ± 4.3	19.9 ± 6.0	18.7 ± 3.3	0.706
Severe hypertrophy >20 mm	16 (42.1%)	6 (46.2%)	10 (40.0%)	0.399
LVOT pressure (mmHg)	67 (3–133)	52 (3–109)	72 (9–133)	0.128
LVOT >30 mmHg	30 (78.9%)	8 (61.5%)	22 (88.0%)	0.09
LVOT >50 mmHg	28 (73.7%)	7 (53.8%)	21 (84.0%)	0.06
ELVH with strain pattern	26 (68.4%)	8 (61.5%)	18 (72.0%)	0.510
Treatment				
Alcohol septal ablation	17 (44.7%)	4 (30.8%)	13 (52.2%)	0.036
PM	3 (7.9%)	3 (23.1%)	0	
Medications only	18 (47.4%)	6 (46.2%)	12 (48.0%)	

LVOT, left ventricular outflow tract; NYHA, New York Heart Association; PM, permanent pacemaker; SCD, sudden cardiac death; LV, left ventricular; ELVH, left ventricular hypertrophy by electrocardiographic criteria.

obstruction (pressure gradient, 109 mmHg). He underwent alcohol septal ablation and the symptoms were alleviated. Later, he died of heart failure at the age of 52 years. His son was also diagnosed with HCM, but his younger daughter and granddaughter were genotype positive–phenotype negative. The Proband *MYBPC3* R597Q was diagnosed at age 66 years, having experienced chest pain. Echocardiography showed that he had severe ASH, non-obstructive type. His eldest son had mild HCM, and his 16-year-old granddaughter had no disease phenotype. Proband *MYBPC3* W1007R was diagnosed at the age of 18 years, after cardiac arrest. Echocardiography showed severe ASH with an LVOT pressure gradient of 74 mmHg. His father was said to have experienced SCD. Proband *TNNI3* E124Q was diagnosed at the age of 48 years after cardiac arrest from which he was successfully resuscitated. Echocardiography showed LV systolic dysfunction (ejection fraction 38%) with apical aneurysm. His father and younger sisters had died suddenly at the ages of 46 and 12 years, respectively. His daughter had asymptomatic HCM. Proband *MYL3* R63C, a 32-year-old man, experienced dyspnea; electrocardiography exhibited an intraventricular conduction defect. Echocardiography demonstrated ASH with an LVOT pressure gradient of 52 mmHg. No information was available for the cause of his father's death. The proband carrying double mutations (*MYH7* R858C and *TNNT2* R286H) had greater LV hypertrophy than his family members who carried a single mutation (either *MYH7* R858C or *TNNT2* R286H) (Fig. 1f), but none of the family members manifested sudden death, lethal arrhythmia, LVOT obstruction (pressure gradient >30 mmHg), or severe heart failure.

Discussion

The study performed sarcomeric gene analysis and cascade screening for family members. The genetic diagnostic rate was 34.2% in the study cohort. Additionally, five novel mutations and a double mutation were identified. The prevalence of sarcomeric gene mutations in the study was lower than the reported prevalence in the US cohort with HCM, the French cohort, and the Japanese cohort, which were 54.2%, 60.6%, and 43.8%,

Table 2
Mutations identified in *MYH7*, *MYBPC3*, *TNNT2*, *TNNI3*, and *MYL3*.

Disease-causing gene	Mutation site	cDNA	Amino acid change		Mutation type	Protein location	Frequency
MYH7	Exon 4	c.533C>T	p.Arg143Trp	(R143W)	Missense	S1	1
	Exon 4	**c.547G>T**	**p.Arg147Ser**	**(R147S)**	**Missense**	**S1**	**1**
	Exon 17	c.2094G>A	p.Arg663His	(R663H)	Missense	Acting-binding	1
	Exon 22	c. 2678C>T	p.Arg858Cys	(R858C)	Missense	S2	1
	Exon 38	c. 5667C>T	p.Thr1854Met	(T1854M)	Missense	LMM	1
	Exon 39	c. 5810G>C	p.Glu1902Gln	(E1902Q)	Missense	LMM	1
MYBPC3	**Exon 18**	**c. 1845G>A**	**p. Arg597Gln**	**(R597Q)**	**Missense**	**C4**	**1**
	Exon 27	c. 2919_2920delCT	p. Pro955ArgfsX95	(P955fs)	Frameshift	Fibronectin type-III 2	1
	Exon 28	c. 3047C>G	p. Gln998Glu	(Q998E)	Missense	C8	1
	Exon 29	**c. 3074T>C**	**p. Trp1007Arg**	**(W1007R)**	**Missense**	**C8**	1
TNNT2	Exon 16	c. 956G>A	p. Arg286His	(R286H)	Missense	C-terminal, binding site of α-TM/cTnC/cTnI	2
TNNI3	**Exon 6**	**c. 513G>C**	**p. Glu124Gln**	**(E124Q)**	**Missense**	**cTnC-binding domain**	**1**
MYL3	**Exon 3**	**c. 280T>C**	**p. Arg63Cys**	**(R63C)**	**Missense**	**EF-hand 1**	**1**

Bold, mutations not previously described.
S1, globular motor domain, subfragment 1; S2: globular motor domain, subfragment 2; LMM, light meromyosin; C: immunoglobulin-like domain numbered consecutively (C4, 8); EF hand, a helix-loop-helix structural domain or motif found in a large family of calcium-binding proteins; α-TM, α-tropomyosin; cTnC, cardiac troponin C.

Table 3
Echocardiographic and electrocardiographic findings at age of diagnosis in index patients carrying mutations.

	Index	LVOT (mmHg)	MWT (mm)	IVST (mm)	PWT (mm)	MV E/A velocity (cm/s)	E/A ratio	Dec (ms)	Mean e' (cm)	Mean E/e' ratio	LVEF (%)	ELVH	Q wave	ST–T	Combination
Single mutation															
MYH7 R143W	H-18	46	18	17	15	82/81	1.02	208	5.9	13.9	72	–	–	–	–
MYH7 R147S	H-07	109	18	17	9	99/126	0.55	158	6.7	14.8	69	+	+	–	+
MYH7 R663H	H-37	86	29	27	14	50/95	0.52	290	3.5	14.3	71	+	–	+	+
MYH7 T1854M	H-25	98	23	20	12	108/66	1.63	225	7.1	15.2	61	+	–	+	+
MYH7 E1902Q	H-32	3	14	14	10	76/99	0.76	225	6.8	11.1	75	+	–	+	+
MYBPC3 R597Q	H-27	18	16	16	12	53/104	0.51	350	4.3	12.3	64	+	–	+	+
MYBPC3 P955fs	H-26	56	22	22	15	128/[a]	[a]	170	8.2	15.6	58	+	–	–	+
MYBPC3 Q998E	H-06	106	22	21	12	75/118	0.64	380	6.2	12.1	52	+	–	+	+
MYBPC3 W1007R	H-35	74	28	27	10	107/51	2.1	200	9.8	10.9	61	+	–	+	+
TNNT2 R286H	H-24	12	24	23	15	110/29	3.8	100	10	11	57	+	–	+	+
TNNI3 E124Q	H-21	18	14	14	11	35/33	1.06	190	5.2	6.7	38	+	–	+	+
MYL3 R63C	H-14	52	16	15	13	82/60	1.37	220	6.2	13.2	58	+	–	–	+
Double mutation															
MYH7 R858C/ *TNNT2* R286H	H-01	15	26	24	10	79/46	1.71	140	5.4	14.6	65	+	–	–	+

LVOT, left ventricular outflow tract pressure gradient; MWT, maximum wall thickness; IVST, interventricular septum; PWT, posterior wall thickness; MV, mitral valve inflow; Dec, deceleration time; mean e', mean of (septal e' + lateral e'); ELVH, left ventricular hypertrophy by electrocardiographic criteria (Sokolow–Lyon >38 mm); ST–T, ST segment depression and T wave inversion; Combination: Sokolow–Lyon and/or abnormal Q and/or ST–T.
[a] No A velocity due to atrial fibrillation.

respectively [11–13]. The lower rate in the present study can be attributed to our enrollment of only symptomatic patients, more sporadic cases (without family history or affected relatives), and relatively older age. The failure to identify genetic mutations in some probands may be due to mutations being present in untested genes or in the non-coding (intron or promoter) regions of the genes screened or due to technical limitations of direct DNA sequencing for large deletion/insertion mutations. In agreement with the previous studies, the two most common genes were *MYH7* and *MYBPC3*, followed by *TNNT2*.

In Taiwan, Ko et al. [14,15], using genetic linkage analysis, reported in 1996 that Taiwanese with HCM might be heterogeneous, but identified only one mutation, *MYH7* R453C, which was associated with a malignant clinical course (coexistence of SCD and end-stage heart failure). Since then, no other sarcomere-related mutations have been reported in Taiwan. Of the eight previously described mutations in the study cohort, *MYBPC3* P955fs has been reported frequently in Caucasian and Asian patients [12,16–20]. It has been confirmed in large families with many affected individuals, and its penetrance has been found to remain incomplete through the fifth decade of life. An in vitro functional study reported that the frameshift truncated mutation causes haploinsufficiency and deranged phosphorylation of the contractile protein [18]. The other mutations (*MYH7* R143W, R663H, R858C, and T1854M; *MYBPC3* Q998E; and *TNNT2* R286H) were confirmed by either cosegregation with affected members in the family or were previously recognized to cause disease in patients with HCM, but the genotype–phenotype expression varied widely between and within families in clinical settings [6,7,11,21–25]. To study the functional alterations of the mutants in the cohort, we used several in silico tools that are designed to predict the pathogenicity type, showing the bioinformatic prediction software could be an adjuvant tool but that the prediction must be interpreted carefully.

Among the five novel mutations in the cohort, the *MYH7* R147S mutation results in an amino acid substitution located in the globular head of the protein and affects the binding sites for ATP, actin, and the essential/regular light chain [26,27]. Probands carrying the mutation *MYH7* R147S presented with HCM, obstructive type, and underwent alcohol septal ablation for relief of symptoms. Both *MYBPC3* R597Q and W1007R mutations result in changes in the charge of the altered amino acid in the immunoglobulin-like C4 and C8 domains [28], but the phenotypes were different in our cohort. The patient with the *MYBPC3* R597Q mutation did not show the phenotype until middle age, whereas the patient with the *MYBPC3* W1007R mutation presented with severe ASH and sudden death at a young age. The *TNNI3* E124Q

Table 4
Predictions from SIFT, PolyPhen, SNAP, and Pmut for the missense mutations.

Gene	Amino acid change	SIFT (score)	PolyPhen (score)	SNAP (reliability index)	Pmut (reliability index)
MYH7	R143W	Damaging (0)	Probably damaging (0)	Non-neutral (2)	Pathological (9)
	R147S	**Damaging (0)**	**Possibly damaging (0.942)**	**Non-neutral (1)**	**Pathological (0)**
	R663H	Tolerated (0.1)	Possibly damaging (0.844)	Neutral (2)	Pathological (3)
	R858C	Damaging (0)	Probably damaging (0.989)	Non-neutral (3)	Pathological (8)
	T1854M	Damaging (0.02)	Probably damaging (0.997)	Non-neutral (0)	Pathological (6)
	E1902Q	Damaging (0.04)	Possibly damaging (0.741)	Neutral (6)	Neutral (1)
MYBPC3	**R597Q**	**Damaging (0.01)**	**Probably damaging (0)**	**Non-neutral (3)**	**Pathological (1)**
	Q998E	Damaging (0)	Probably damaging (0.992)	Non-neutral (1)	Neutral (6)
	W1007R	**Damaging (0)**	**Probably damaging (0)**	**Non-neutral (5)**	**Pathological (9)**
TNNT2	R286H	Damaging (0)	Probably damaging (0)	Non-neutral (3)	Pathological (6)
TNNI3	**E124Q**	**Damaging (0.01)**	**Probably damaging (0.985)**	**Non-neutral (1)**	**Neutral (4)**
MYL3	**R63C**	**Damaging (0)**	**Probably damaging (1.0)**	**Non-neutral (1)**	**Pathological (8)**

Bold, mutations not previously described.

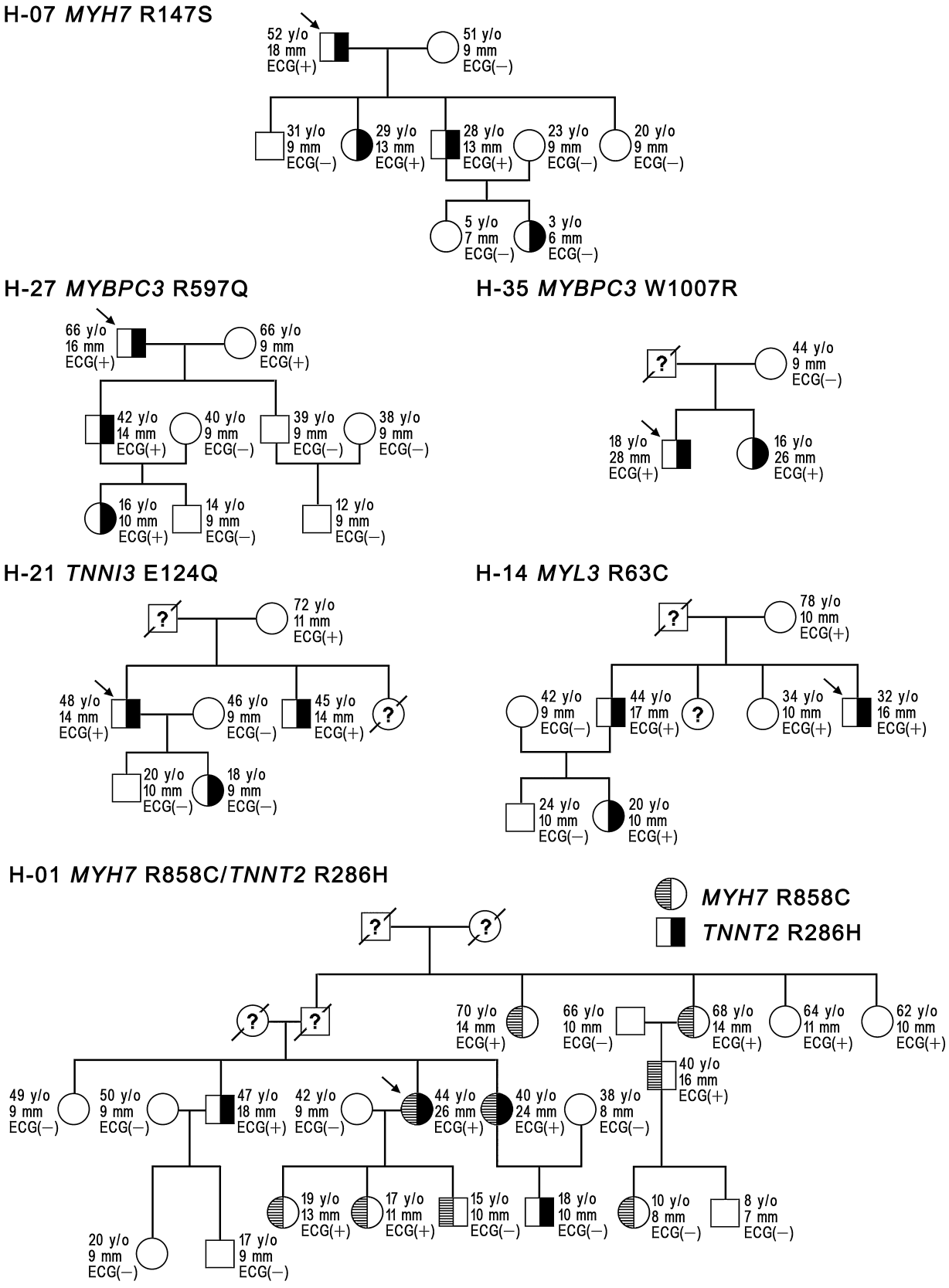

Fig. 1. Pedigree and mutation analysis of families with either novel mutations or double mutations. Circles indicate female family members; squares, male family members; open symbols, unaffected family members; arrows, the proband; symbols with a slash mark, those who have died; question marks, unknown phenotype; and plus signs, presence of the hypertrophic cardiomyopathy phenotype. The half-filled symbols indicate heterozygosity. The phenotypes, including age, maximal ventricular wall thickness by echocardiography, and ECG are presented. ECG(−): normal finding in ECG, ECG(+): presence of Sokolow–Lyon and/or Q wave and/or ST–T. ECG, electrocardiogram.

mutation is located in exon 6, which is the site of interaction with the troponin C-binding domains, near the actin-binding domain. In contrast to previous data, the *TNNI3* mutations appeared to cluster in exons 7 and 8, and not in exon 6 [29]. *TNNI3* E124Q family members showed sudden death, high penetrance, and severe LV dysfunction; these variable clinical features were similar to *TNNI3* Lys 183 deletion mutation reported by Kokado et al. in Japan [30]. End-stage HCM can be seen in around 5% of HCM cases, with highly unfavorable complications [31,32]. Whether HCM caused by *TNNI3* E124Q mutation is malignant prognosis deserves greater investigation. To date, only 12 HCM-causing mutations have been described in *MYL3* [33–35]. The *MYL3* R63C mutation is predicted to be located in the α-helix of the first EF-hand domain and to affect a Ca^{2+}-binding motif. Amino acid alignment across various species has shown that arginine 63 is highly conserved in *MYL3* [35,36]. In the study cohort, the proband *MYL3* R63C phenotype exhibited late onset, dyspnea, an intraventricular conduction defect, and obstructive HCM.

One proband had two mutations (*MYH7* R858C and *TNNT2* R286H) (Fig. 1, H-01). The two missense mutations have been reported before, but this is the first reported patient carrying them simultaneously. In agreement with the previous study [11], the proband and one family member carrying the double mutation experienced a dosage effect associated with severe LV hypertrophy, while others carrying a single mutation did not. The emerging data suggested that more than one HCM-associated sarcomere mutation could be associated with increased disease severity. With continuing improvements, targeted next-generation sequencing allows simultaneous screening of multiple genes [37]. It will provide a more comprehensive mutation screening to identify high-risk patients who would benefit from earlier treatment and strategies in the future.

The following limitations of the study should be acknowledged. First, clinical evaluation was not available for some subjects who had SCD and could not be evaluated before their death. Second, prospective follow-up studies of clinically unaffected mutation carriers are required to determine the precise age of disease onset as well as the true disease penetrance. Third, genes linked to HCM and with less evidence for pathogenicity, such as those encoding the α-myosin heavy chain, titin, muscle LIM protein, telethonin, vinculin, and junctonphilin 2, were not analyzed. Furthermore, non-sarcomeric protein genes (*GLA*, *GAA*, *LAMP2*, and *PRKG2*) were not analyzed.

In conclusion, the study identified 13 HCM pedigrees, including 5 carrying novel mutations and one double mutation from ethnic Chinese living in Taiwan. The three most commonly mutated genes were *MYH7*, *MYBPC3*, and *TNNT2*. These results, together with genetic counseling, could lead to earlier diagnosis and better management of family members at risk of HCM.

Conflict of interest

The authors have no conflicts of interest to disclose.

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