

Clinical Features and Outcome of Hypertrophic Cardiomyopathy Associated With Triple Sarcomere Protein Gene Mutations

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- Objectives** The aim of this study was to describe the clinical profile associated with triple sarcomere gene mutations in a large hypertrophic cardiomyopathy (HCM) cohort.
- Background** In patients with HCM, double or compound sarcomere gene mutation heterozygosity might be associated with earlier disease onset and more severe outcome. The occurrence of triple mutations has not been reported.
- Methods** A total of 488 unrelated index HCM patients underwent screening for myofilament gene mutations by direct deoxyribonucleic acid sequencing of 8 genes, including myosin binding protein C (*MYBPC3*), beta-myosin heavy chain (*MYH7*), regulatory and essential light chains (*MYL2*, *MYL3*), troponin-T (*TNNT2*), troponin-I (*TNNI3*), alpha-tropomyosin (*TPM1*), and actin (*ACTC*).
- Results** Of the 488 index patients, 4 (0.8%) harbored triple mutations, as follows: *MYH7*-R869H, *MYBPC3*-E258K, and *TNNI3*-A86fs in a 32-year-old woman; *MYH7*-R723C, *MYH7*-E1455X, and *MYBPC3*-E165D in a 46-year old man; *MYH7*-R869H, *MYBPC3*-K1065fs, and *MYBPC3*-P371R in a 45-year old woman; and *MYH7*-R1079Q, *MYBPC3*-Q969X, and *MYBPC3*-R668H in a 50-year old woman. One had a history of resuscitated cardiac arrest, and 3 had significant risk factors for sudden cardiac death, prompting the insertion of an implantable cardioverter-defibrillator in all, with appropriate shocks in 2 patients. Moreover, 3 of 4 patients had a severe phenotype with progression to end-stage HCM by the fourth decade, requiring cardiac transplantation (n = 1) or biventricular pacing (n = 2). The fourth patient, however, had clinically mild disease.
- Conclusions** Hypertrophic cardiomyopathy caused by triple sarcomere gene mutations was rare but conferred a remarkably increased risk of end-stage progression and ventricular arrhythmias, supporting an association between multiple sarcomere defects and adverse outcome. Comprehensive genetic testing might provide important insights to risk stratification and potentially indicate the need for differential surveillance strategies based on genotype.
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Hypertrophic cardiomyopathy (HCM) is the most common genetic cardiac disease, characterized by heterogeneous

morphologic expression and clinical course (1,2). Mutations in genes coding for myofilament contractile proteins of the cardiac sarcomere represent the most common genetic subtype of HCM, with a prevalence of 30% to 65% in cohort studies (3–7). Double or compound heterozygosity

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(i.e., patients with 2 distinct mutations in the same or in different sarcomere genes) has been described in 3% to 6% of consecutively screened cohorts and has been associated with earlier onset and more severe clinical profile, compared with single mutation HCM (8–12). To our knowledge, HCM associated with triple sarcomere mutations has not previously been reported. In the present study, we describe

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4 pedigrees from 3 HCM referral centers, in which each index patient harbored 3 distinct mutations in sarcomere genes.

Methods

Patient selection. A total of 488 unrelated index patients with a confirmed clinical diagnosis of HCM from 3 referral centers (Florence, Boston, Sydney) underwent systematic screening for mutations in 8 myofilament genes, in the period January 2002 to February 2009 (4). The diagnosis, based on the standard accepted definition for HCM, consisted of 2-dimensional echocardiographic identification of a hypertrophied, nondilated left ventricle (LV), in the absence of another cardiac or systemic disease capable of producing the magnitude of ventricular hypertrophy evident (1,2,13).

Mutational analysis. Patients were screened for mutations in the protein-coding exons and splice sites of 8 myofilament genes, including myosin binding protein C (*MYBPC3*), thick filament proteins (beta-myosin heavy chain [*MYH7*] and the regulatory and essential light chains [*MYL2* and *MYL3*]), and thin filament proteins (troponin-T [*TNNT2*], troponin-I [*TNNI3*], alpha-tropomyosin [*TPM1*], and alpha-actin [*ACTC*]). Direct deoxyribonucleic acid (DNA) sequencing was employed with either ABI-Prism 3730 (Applied Biosystems, Foster City, California) as previously described (4) or the HCM CardioChip platform (Laboratory for Molecular Medicine, Cambridge, Massachusetts). This latter platform includes a combination of direct sequencing of *MYBPC3* and oligonucleotide hybridization-based DNA sequencing of the coding regions and splice sites of the *MYH7*, *MYBPC3*, *TNNT2*, *TNNI3*, *TPM1*, *ACTC*, *MYL2*, *MYL3*, *LAMP2*, *PRKAG2*, and *GLA* genes with a custom-designed Affymetrix GeneChip (Affymetrix, Santa Clara, California) platform. Every variant identified was confirmed by direct sequencing and, whenever possible, by restriction enzyme digestion (4). Novel mutations were considered potentially disease-causing only if they were absent in at least 300 unrelated chromosomes from adult, ethnicity-matched, healthy control subjects and produced a change in a highly conserved residue among species and isoforms (14).

Statistical methods. The prevalence of end-stage progression among our patients with triple mutations was compared with that of the overall HCM population undergoing genetic screening at our institutions (n = 488) with the Fisher exact test.

Results

Of the 488 index patients, 251 were found to harbor at least 1 putative HCM-susceptibility gene mutation (51%). Of these, 223 had single mutations (46%) and 24 had double mutations (5%). In addition, 4 patients (0.8%) carried 3 distinct mutations, involving *MYH7*, *MYBPC3*, and *TNNI3* (Table 1). Three of the 4 index patients with triple mutations showed early onset of severe HCM phenotype asso-

ciated with marked symptoms, ventricular arrhythmias, and a progressive clinical course culminating in classic end-stage manifestations. These data indicate that the presence of triple mutations confers a relative risk of 14 (95% confidence interval: 5 to 18; p = 0.001) for development of an end-stage phenotype.

Patient #1. A 32-year-old woman (II-1, Fig. 1) was diagnosed with HCM at the age of 18 years after presentation with dyspnea and exertional angina. At the time, she had no family history of cardiovascular disease or sudden cardiac death. An echocardiogram at presentation showed extreme LV hypertrophy (maximum LV wall thickness 32 mm) and severe LV outflow tract obstruction (peak gradient 85 mm Hg) at rest. At age 24 years her maximum LV wall thickness had regressed to 27 mm, LV obstruction had spontaneously disappeared, and there were signs of severe diastolic dysfunction with left atrial dilation (Fig. 2). Cardiac magnetic resonance imaging showed substantial late-gadolinium enhancement in the thinned portion of the basal septum, compatible with replacement fibrosis. During follow-up she developed progressive deterioration of systolic and diastolic function, with recurrent angina, episodes of acute congestive heart failure, and recurrent atrial fibrillation. A second cardiac magnetic resonance imaging at age 30 years showed marked increase in delayed contrast enhancement (Fig. 2), and positron emission tomography scan showed severe microvascular dysfunction (average myocardial flow after dipyridamole infusion 1.31 ml/g/min; normal values ≥ 2.0 ml/g/min). She underwent successful transcatheter ablation of atrial fibrillation and has remained in sinus rhythm with satisfactory hemodynamic balance on amiodarone, carvedilol, angiotensin-converting enzyme inhibitors, and loop diuretics. A prophylactic implantable cardioverter-defibrillator (ICD) with biventricular pacing capability was subsequently implanted.

Genetic analysis revealed heterozygous mutations in 3 distinct sarcomere genes, including a missense mutation in *MYH7* (R869H), a splice site mutation in *MYBPC3* (E258K), and a frameshift mutation in *TNNI3* (A86fs). Both *MYH7*-R869H and *MYBPC3*-E258K have been previously reported in HCM (Genomics of Cardiovascular Development, Adaptation, and Remodeling; NHLBI Program for Genomic Applications, Harvard Medical School). Family screening revealed the *MYBPC3*-E258K and *TNNI3*-A86fs mutations in the proband's father (I-2, Fig. 1), a 58-year-old gentleman who had always been active and totally asymptomatic. His echocardiogram showed definite HCM, with LV hypertrophy prevalently localized at the apex and preserved LV function (Fig. 1). The proband's mother (I-1, Fig. 1), a 52-year-old woman with a history of mild

Abbreviations and Acronyms

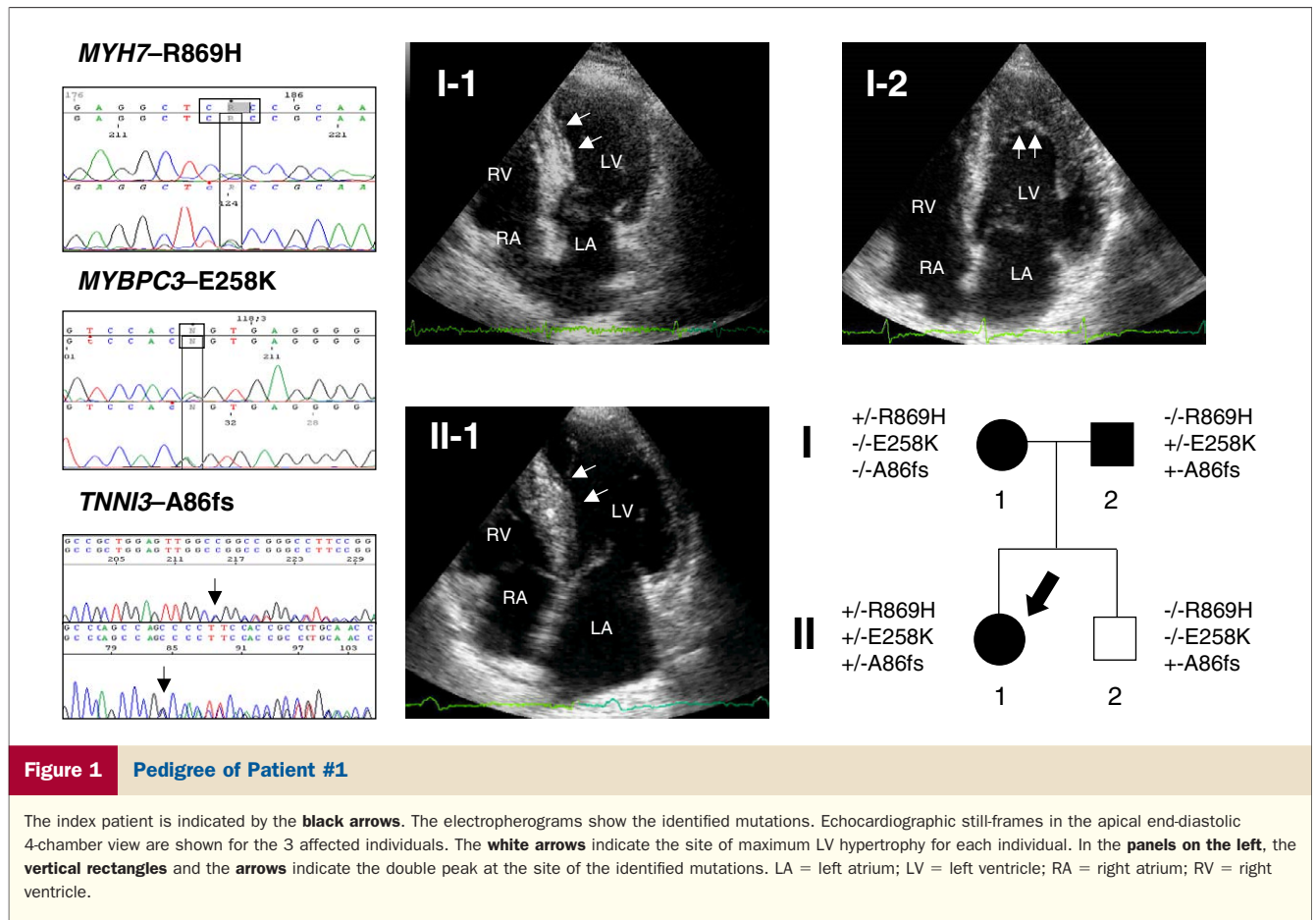
DNA	= deoxyribonucleic acid
ECG	= electrocardiogram
HCM	= hypertrophic cardiomyopathy
ICD	= implantable cardioverter-defibrillator
LV	= left ventricular

Table 1 Clinical Features of the Study Patients and Genotype-Positive Family Members

Pedigree	Individual	Sex	Relation to Index Case	Genotype			Age at Enrollment (yrs)	Age at Last Evaluation (yrs)	Echocardiographic Features					
				MYH7	MYBPC3	TNNI3			Evidence of HCM	Max LV Thickness (mm)	Site	ECG Findings	Symptoms	Events/Outcome
#1	II-1	F	—	R869H	E258K	A86fs*	18	32	Yes	32	Septum	Diffuse repolarization abnormalities, LVH	Dyspnea, palpitations, angina	End-stage, HF, ICD
#1	II-2	M	Brother	—	—	A86fs*	28	30	No	10	—	Normal	None	None
#1	I-1	F	Mother	R869H	—	—	52	53	Yes	16	Septum	Atrial conduction delay	Mild dyspnea	None
#1	I-2	M	Father	—	E258K	A86fs*	58	59	Yes	19	Apex	Diffuse T-wave inversion, LVH	None	None
#2	II-4	M	—	E1455X*	R273C	E165D*	29	46	Yes	17	Septum	LVH, LVS	Progressive dyspnea, angina	End-stage, HF, ICD
#2	II-3	F	Sister	—	—	E165D*	57	58	No	8	—	Normal	None	None
#2	I-1	M	Father	—	R273C	—	80	80	No	9	—	Normal	None	None
#2	I-2	F	Mother	E1455X*	—	E165D*	76	78	Yes	16	Septum	LVH, LVS	Mild dyspnea	None
#2	III-4	F	Niece	—	—	E165D*	35	38	No	8	—	Normal	None	None
#2	III-5	M	Son	E1455X*	—	E165D*	17	19	Yes	12	Septum	Small inferior Q waves	None	None
#3	II-5	F	—	R869H	K1065fs	P371R*	24	45	Yes	36	Septum	LVH, T-wave inversion	Progressive dyspnea	Cardiac arrest, ICD, subsequent end-stage
#3	II-2	F	Sister	Unknown	K1065fs†	P371R*†	—	29	Yes	28	Septum	Unknown	Unknown	Died of stroke (likely cardioembolic)
#3	I-1	M	Father	—	K1065fs	P371R*	61	69	Yes	19	Septum	Nonspecific ST-T-wave changes	None	None
#3	III-1	M	Nephew	—	K1065fs	P371R*	19	21	Yes	16	Septum	Normal	None	None
#4	II-2	M	—	R1079Q	—	Q969X	47	54	Yes	16	Septum	Left axis deviation, nonspecific ST-T-wave changes	Atypical chest pain	None
#4	II-3	F	Sister	R1079Q	R668H	Q969X	48	50	Yes	19	Septum	Normal, NSVT on Holter	Atypical chest pain	ICD
#4	III-5	F	Niece	R1079Q	—	Q969X	33	34	No	8	—	Normal	None	None
#4	III-7	F	Niece	R1079Q	R668H	—	30	31	No	9	—	Normal	None	None
#4	III-8	F	Niece	—	—	Q969X	29	30	No	8	—	Normal	None	None

*Novel mutation. †Obligate carrier.

ECG = electrocardiographic; HCM = hypertrophic cardiomyopathy; HF = (congestive) heart failure; ICD = implantable cardioverter-defibrillator; LV = left ventricular; LVH = left ventricular hypertrophy; LVS = left ventricular strain; NSVT = nonsustained ventricular tachycardia.



dyspnea on effort, had mild asymmetric septal hypertrophy associated with the *MYH7*-R869H mutation. The proband's brother, age 28 years (II-2, Fig. 2), only had the *TNNI3*-A86fs mutation and was clinically unaffected, with normal electrocardiogram (ECG) and echocardiographic findings.

Patient #2. A 46-year-old man was diagnosed at the age of 29 years due to mild exertional dyspnea (Fig. 3). At the time, he had nonobstructive HCM with LV chamber dilation and systolic function at the lower limits of normal (ejection fraction 56%). In the following years he developed marked LV remodeling with progressive systolic dysfunction and wall-thinning in conjunction with severe symptoms of congestive heart failure (Fig. 4). A positron emission tomography scan at age 33 years showed marked microvascular dysfunction (average myocardial flow after dipyridamole infusion 1.06 ml/g/min (normal range >2.00 ml/g/min in this age group). At age 39 years he received an ICD, which repeatedly intervened to terminate runs of sustained ventricular tachycardia. At age 43 years he required cardiac transplantation due to refractory heart failure. His LV cavity measured 238 ml in diastole, and his ejection fraction was 28%. After transplantation he rapidly recovered and is now well.

Genetic analysis revealed 3 missense mutations, of which 2 were in *MYH7* (R723C and E1455X) and 1 in *MYBPC3*

(E165D). Of these mutations, *MYH7*-R723C has been described previously (5), whereas the premature truncation, nonsense mutation in beta myosin heavy chain (*MYH7*-E1455X) and *MYBPC3*-E165D are novel. Notably, although nonsense mutations in *MYH7* have not been demonstrated to be pathogenic in HCM, the 2 *MYH7* mutations present in this patient were inherited in trans, indicating that he had no normal beta-myosin heavy chain protein. Both novel mutations were absent in 300 unrelated chromosomes from healthy control subjects; in addition *MYBPC3*-E165D changes a residue that is highly conserved among species. The proband's mother (I-2, Fig. 3) was compound heterozygote possessing both *MYH7*-E1455X and *MYBPC3*-E165D but exhibited mild, nonobstructive HCM; the proband's father (I-1, Fig. 3) had the *MYH7*-R723C mutation but was clinically unaffected at age 80 years. The proband's sister (II-3, Fig. 3), age 57 years, inherited only her mother's *MYBPC3*-E165D mutation and was also clinically unaffected. His brother (II-2, Fig. 3) was genotype-negative. The proband's son (III-5, Fig. 3), age 19 years, carried the *MYH7*-E1455X and *MYBPC3*-E165D mutations. He was asymptomatic, but his echocardiogram showed mild asymmetric hypertrophy of the basal septum and anterior papillary muscle, consistent with mild HCM.

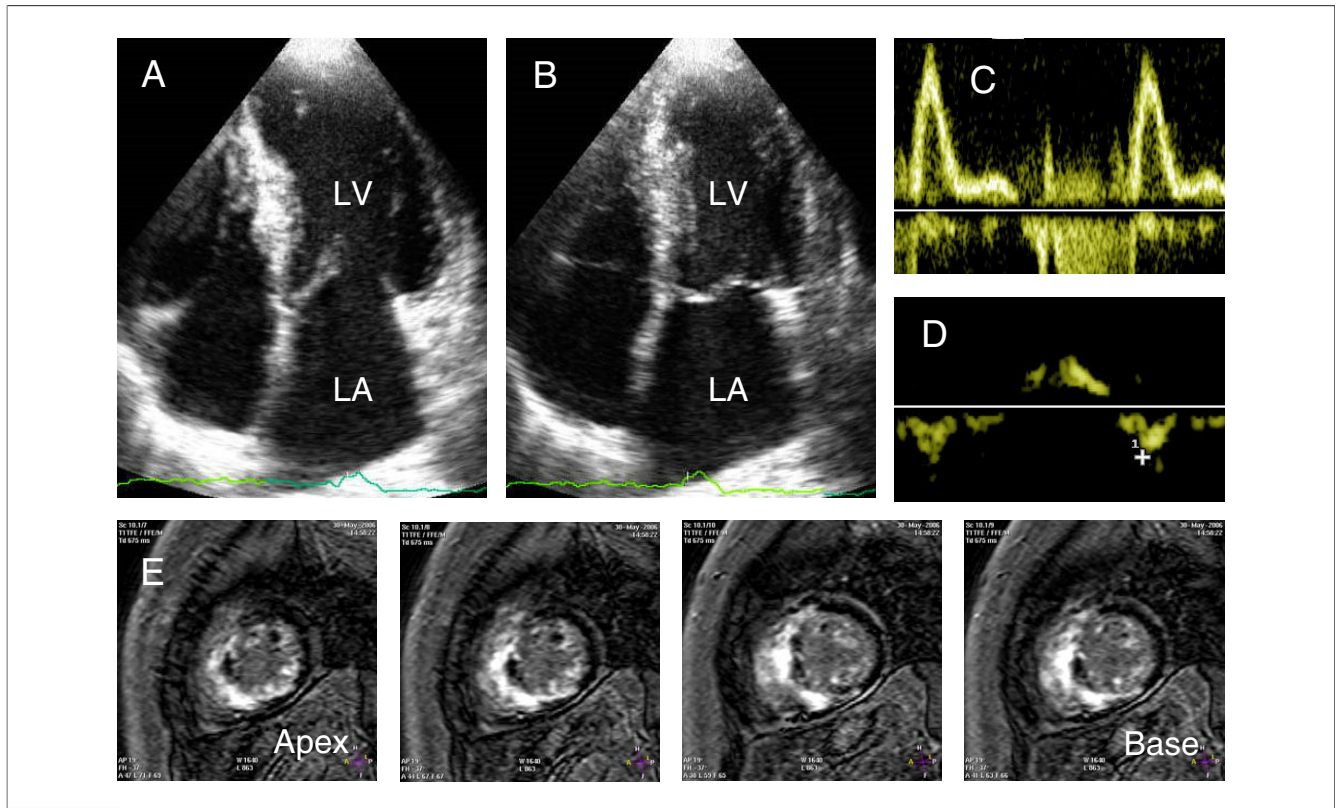


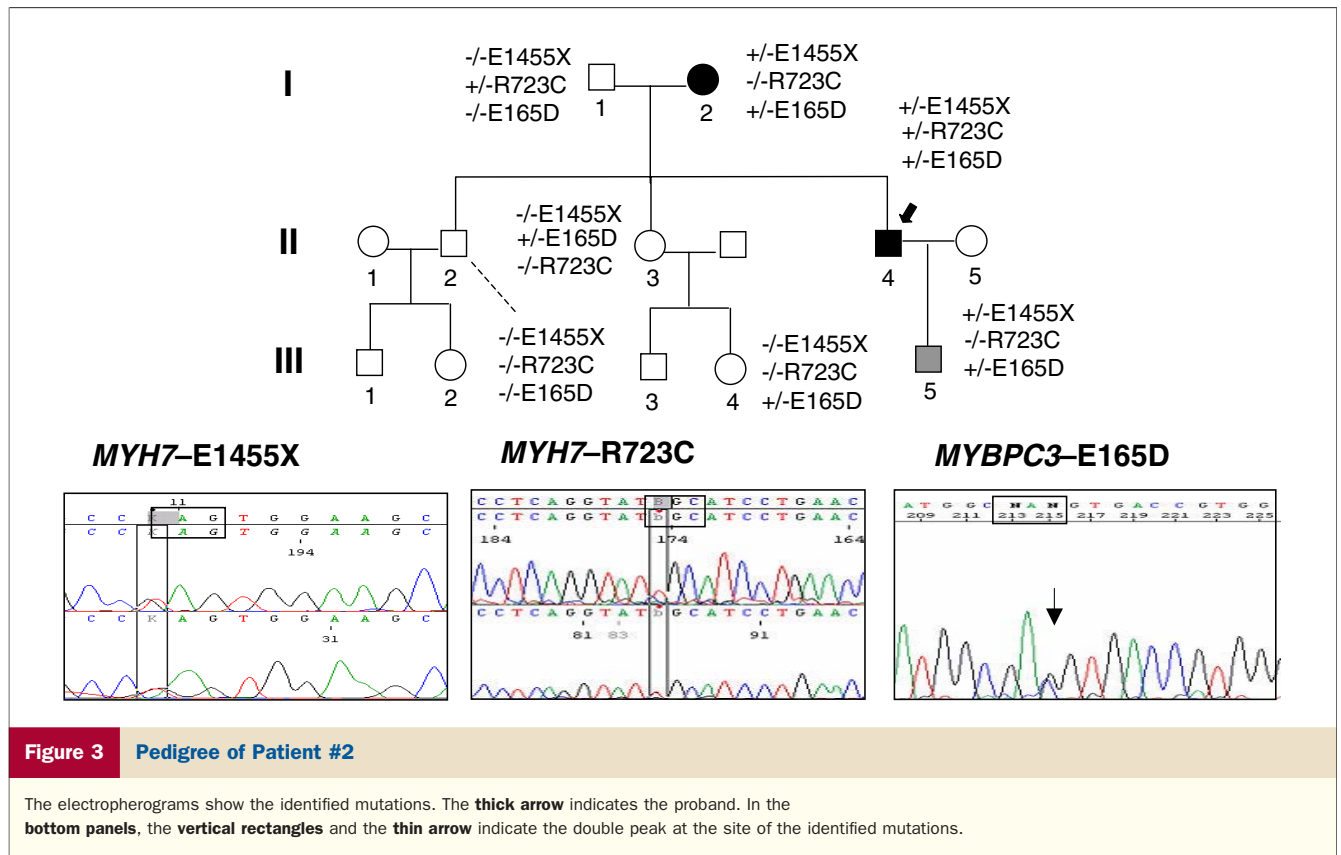
Figure 2 Evidence of Severe LV Dysfunction and Fibrosis in Patient #1

Echocardiographic apical 4-chamber view at end-diastole (A) and end-systole (B) showing mild left ventricular (LV) cavity dilation with reduced ejection fraction, a bright, akinetic ventricular septum, and marked left atrial (LA) dilation. Pulsed-wave Doppler showing a restrictive mitral flow pattern (C) associated with severe early diastolic tissue Doppler velocity reduction (D) E' 5 cm/s). Cardiac magnetic resonance imaging end-diastolic short-axis images demonstrate extensive transmural late gadolinium enhancement in the ventricular septum extending into the posterior free wall (E).

Patient #3. A 45-year-old woman was diagnosed with HCM at the age of 24 years after resuscitation from cardiac arrest due to documented ventricular fibrillation (Fig. 5). An echocardiogram showed severe asymmetric LV hypertrophy with a maximum thickness of 36 mm at the basal septum (Fig. 6), elongated mitral leaflets without evidence of outflow obstruction, and normal LV systolic function. During follow-up her echocardiograms showed progressive septal thinning and a decline in LV function, with a recent evaluation showing a basal septum of 18 mm (Fig. 6) and an LV ejection fraction of 42%. This coincided with a clinical deterioration and development of exertional dyspnea, despite significant medical therapy with carvedilol, angiotensin receptor blockers, and diuretics. An ICD was placed for secondary prevention after her cardiac arrest, repeatedly replaced over the years, and recently upgraded to perform biventricular pacing. Of note, cardiac resynchronization has led to significant subjective improvement in quality of life and exercise tolerance. During follow-up, however, she received 2 appropriate ICD discharges due to rapid sustained ventricular tachycardia.

Genetic screening showed 1 mutation in MYH7 (R869H) and 2 in MYBPC3 (K1065fs and P371R). Both MYBPC3-K1065fs and MYH7-R869H had been previously reported

(Genomics of Cardiovascular Development, Adaptation, and Remodeling; NHLBI Program for Genomic Applications, Harvard Medical School). Conversely, MYBPC3-P371R was novel but was absent in 300 unrelated chromosomes from healthy control subjects and produced a change in a highly conserved residue. Co-segregation studies in this family showed that K1065fs and P371R are pathogenic when inherited together, as in the proband's nephew (III-1, Fig. 5). Of note, another unrelated index case in the Florence cohort has been found to have both K1065fs and P371R. The proband's father (I-1, Fig. 5), age 69 years, had a family history of premature sudden death and was found to have a mild HCM phenotype, characterized by maximum septal thickness of 19 mm, with preserved LV function and excellent exercise tolerance (maximum oxygen consumption was 32 ml/min/kg). He carries both MYBPC3-K1065fs and MYBPC3-P371R. One of the proband's sisters (II-2, Fig. 5) died suddenly at the age of 31 years, possibly from cardioembolic stroke. Obligate affected status for both MYBPC3 mutations could be inferred, due to their presence in her only child. This individual, age 21 years (III-1, Fig. 5), has a mild form of nonobstructive HCM (maximum LV wall thickness 16 mm) with preserved LV function and is asymptomatic.



Patient #4. A 50-year-old woman (II-3, Fig. 7) was diagnosed with HCM at the age of 48 years as part of clinical screening of her family. In contrast to the other 3 pedigrees shown, she was not the index case in her family, because the first patient identified was her brother (II-2, Fig. 7). On presentation, the patient had mild symptoms of atypical chest pain for several years. Her echocardiogram showed moderate nonobstructive HCM with a maximum LV wall thickness of 19 mm and normal LV dimensions and systolic function. Although she had no history of syncope, 24-h ambulatory ECG monitoring identified several runs of nonsustained ventricular tachycardia (HR >140 beats/min, longest run of 7 beats) (Fig. 7). She had an ICD implanted and has had no shocks delivered at 6-month follow-up.

Genetic analysis identified 3 missense mutations, of which 1 was in the beta myosin heavy chain (*MYH7*-R1079Q), and 2 were in cardiac myosin binding protein C (*MYBPC3*-Q969X and R668H). All 3 mutations have been identified in isolation in previous HCM families or probands as single, presumed pathogenic gene mutations (Genomics of Cardiovascular Development, Adaptation, and Remodeling; NHLBI Program for Genomic Applications, Harvard Medical School). In contrast to patient #3, who had *MYBPC3* mutations residing on the same allele, these mutations were inherited in trans, as demonstrated by the absence of the R668H mutation in Patient II-2 (Fig. 7).

Of note, that these 2 *MYBPC3* mutations are in trans is also confirmed by their separate transmission in the patient's offspring (Fig. 7).

Subsequent clinical and genetic screening of first-degree relatives revealed that the 3 daughters of the patient (III-5, III-7, III-8, Fig. 7)—who are ages 33, 30, and 29 years, respectively—have inherited either 1 or 2 of the 3 mutations identified in their mother; nevertheless, all are asymptomatic and show normal ECG and echocardiograms. The patient's brother (the family proband, II-2, Fig. 7), age 54 years, was diagnosed previously with mild nonobstructive HCM (maximum LV wall thickness 16 mm), and is currently asymptomatic. Genetic screening was positive for 2 of the sister's 3 mutations (Fig. 7).

Discussion

Genetic testing in a large cohort of 488 probands with HCM allowed the identification of rare triple sarcomere gene mutations in 4 patients (i.e., a 0.8% prevalence), including 1 with double *MYH7* and a single *MYBPC3* mutation, 2 with a single *MYH7* and double *MYBPC3* mutations, and 1 with single mutations in *MYH7*, *MYBPC3*, and *TNNI3*. To our knowledge, this is the first report of HCM caused by 3 independent genetic defects. Remarkably, 3 of the 4 patients with triple mutations (75%) developed an end-stage phenotype with severe LV dysfunc-

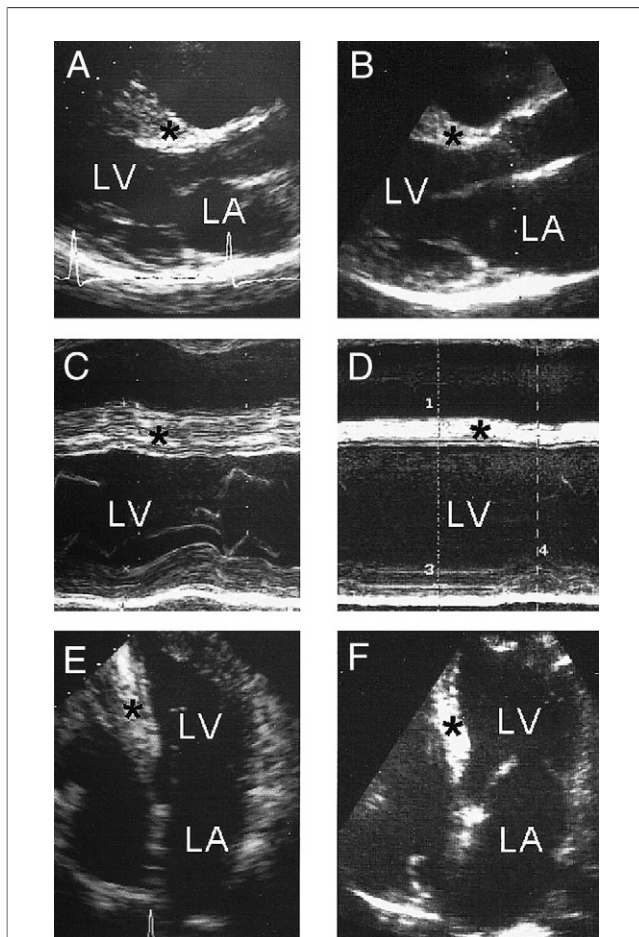


Figure 4 Evidence of Disease Progression in Patient #2

Stop frames of echocardiograms obtained at the time of the positron emission tomography scan (age 33 years; **A, C, and E**) and at final evaluation (age 42 years; **B, D, and F**). Comparison of the 2 echocardiograms shows progression of left ventricular (LV) cavity enlargement and systolic impairment, with regression of septal hypertrophy. (**A to D**) Parasternal long-axis view. (**E and F**) Apical 4-chamber view. *Interventricular septum. LA = left atrium. Reproduced, with permission, from Olivetto et al. (24).

tion. These 3 patients had originally presented with severe LV hypertrophy by age 25 years. In the next 10 to 20 years, however, they developed marked cardiac remodelling characterized by restrictive physiology, atrial dilation, and systolic dysfunction associated with progressive LV wall thinning and fibrosis (15,16); 1 patient required cardiac transplantation (17). By comparison, only 29 of 488 or 6% of the overall genotyped population with HCM seen at our centers developed end-stage disease, similar to the prevalence in other reports (17,18). This suggests that triple mutations confer a 14-fold increase in risk for the development of end-stage disease. Furthermore, all 4 probands with triple mutations had significant ventricular arrhythmias, prompting the implantation of a defibrillator for primary or secondary prevention of sudden cardiac death. Notably, 1 patient had been resuscitated from cardiac arrest, and

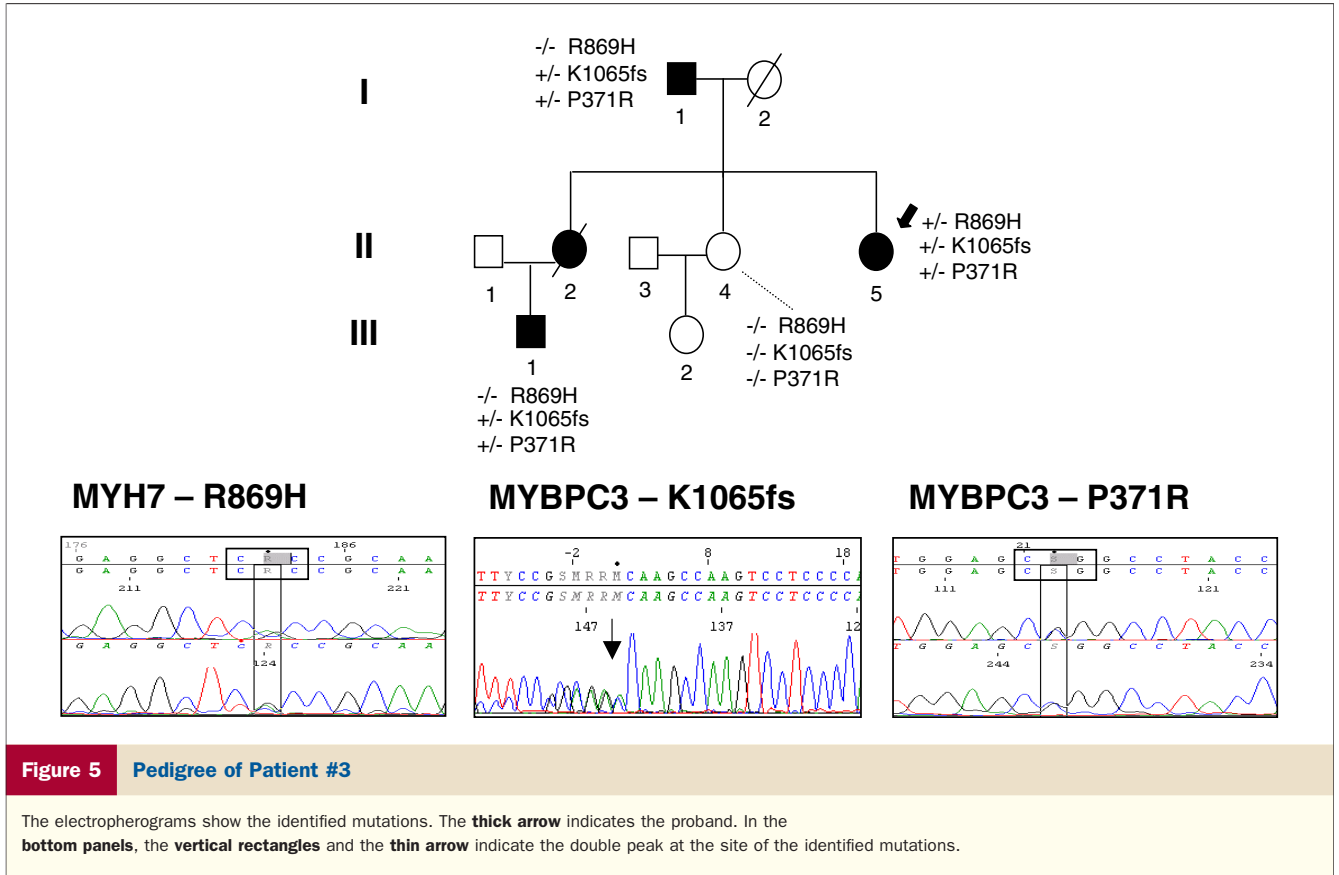
another received multiple appropriate interventions after ICD insertion (15).

Overall, the clinical course of HCM patients with triple mutations strongly supports the concept that multiple sarcomere defects might be associated with more severe clinical phenotype and disease course (8–12,19). The *TNNI3*-203/*MHC*-403 double-mutant mouse model recently reported by Tsoutsman et al. (10), buttresses the concept of a gene dosage effect resulting in more severe clinical phenotypes (11,12,20,21). In this model, although each mutation by itself was linked to a hypertrophic phenotype, the presence of both mutations rapidly led to LV dilation, severe heart failure, and premature death (10), echoing the development of end-stage HCM in our cohort.

Comprehensive sarcomere mutational screening might provide, on the basis of the present findings, important clues to risk stratification and potentially indicate the need for differential surveillance strategies based on genotype. Young patients with multiple mutations might benefit from close clinical and imaging follow-up to allow timely recognition of pending end-stage progression (18). In the presence of an initial decline in systolic function, LV wall thinning, or progression of intramyocardial fibrosis, prompt initiation of angiotensin-converting enzyme inhibition or angiotensin receptor blockade might be beneficial to mitigate further adverse remodeling and should be considered before overt systolic dysfunction has ensued (1,2). Furthermore, patients with more than 1 HCM-causing mutation might require heightened attention with regard to sudden death risk, by virtue of a potentially increased susceptibility to ventricular arrhythmias (15,22).

A plausible explanation of the adverse consequences of complex genotypes is that multiple abnormal myofilament proteins might result in more profound derangement of sarcomere mechanics, myocardial energetics, and cardiomyocyte dysfunction (23). In addition, other pathophysiological mechanisms might intervene, such as greater impairment of microvascular function due to adverse remodeling of the coronary arterioles, leading to recurrent myocardial ischemia and replacement fibrosis (24,25). Two of our patients with triple mutations had evidence of severe microvascular dysfunction and blunted myocardial perfusion (24,25) preceding the development of LV wall thinning and systolic dysfunction, consistent with this hypothesis. In the patient who was subsequently transplanted, we were able to document that both thinning and functional impairment were secondary to widespread fibrotic replacement of the myocardium (18).

Our pedigrees highlight the complexity inherent to the HCM disease process and challenge conventional wisdom regarding the real clinical impact of single sarcomere gene mutations. For example, although most of our patients with triple mutations exhibited severe phenotype and progressive disease, Patient #4 had modest LV hypertrophy and mild symptoms at age 50 years. Furthermore, several adult



relatives of the 4 index patients had mild or no disease expression, despite carrying double mutations themselves. Such a discrepancy suggests that certain DNA variants might not be capable of causing disease in isolation but potentially exert modifying effects on disease expression, in combination with other mutations (10,12,26,27). The demonstration of such a hypothesis is hindered by the objective

difficulty, inherent to all genetic studies in HCM, of proving which of the identified sequence variants are truly pathogenic and to what extent (12,14). However, the striking phenotypic expression and markedly increased prevalence of end-stage remodelling observed in our cohort indicate that the multiplicity of variants importantly contributed to disease pathogenesis and clinical outcome.

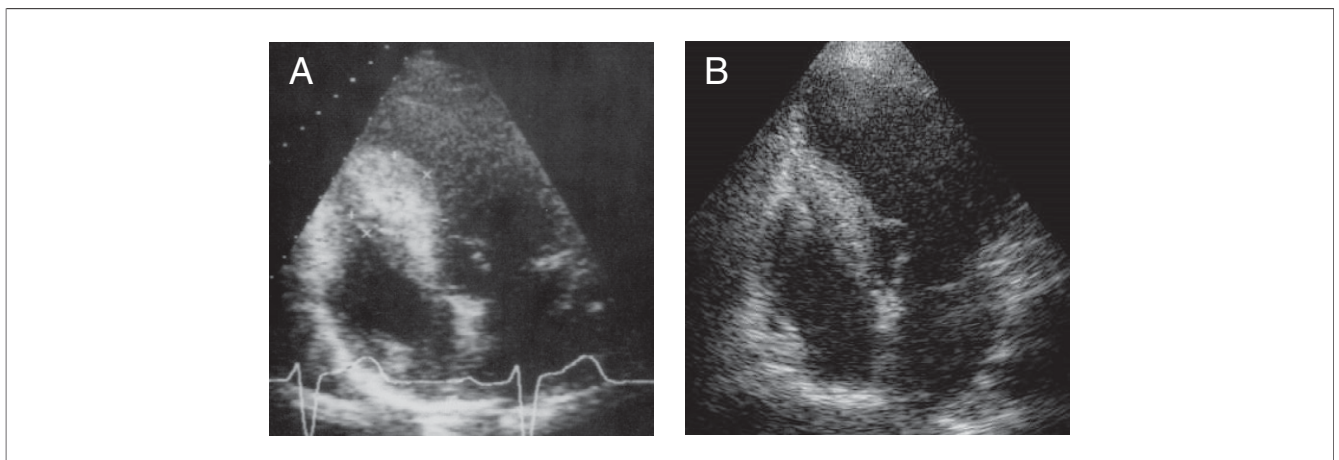
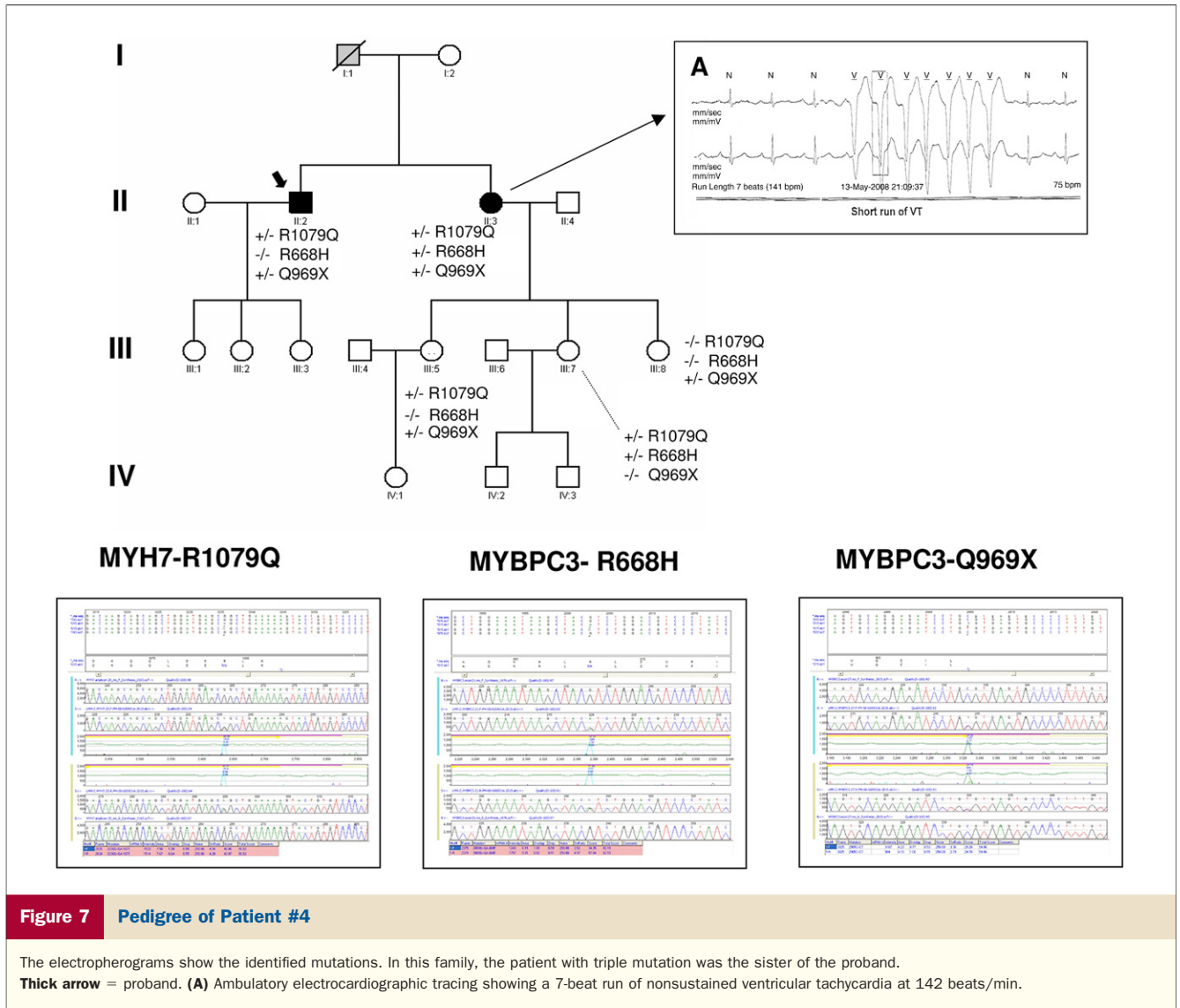


Figure 6 Evidence of Disease Progression in Patient #3

Stop frames of echocardiographic 4-chamber view at end-diastole at age 28 (A) and 45 years (B). Comparison of the 2 echocardiograms shows progression of left ventricular cavity enlargement with marked regression of septal hypertrophy.



Conclusions

HCM associated with triple sarcomere gene mutations is rare but often associated with adverse outcomes, including a remarkably increased risk of developing end-stage disease progression and high prevalence of ventricular arrhythmias. These findings support the theory that multiple sarcomere defects confer an increased risk of disease progression and adverse outcome in HCM. Therefore, comprehensive genetic testing might provide important insights into risk stratification and potentially indicate the need for differential surveillance strategies based on genotype.

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REFERENCES

1. Maron BJ. Hypertrophic cardiomyopathy: a systematic review. *JAMA* 2002;287:1308–20.
2. Maron BJ, McKenna WJ, Danielson GK, et al. American College of Cardiology/European Society of Cardiology clinical expert consensus document on hypertrophic cardiomyopathy. *J Am Coll Cardiol* 2003; 42:1687–713.
3. Van Driest SL, Ommen SR, Tajik AJ, Gersh BJ, Ackerman MJ. Yield of genetic testing in hypertrophic cardiomyopathy. *Mayo Clin Proc* 2005;80:739–44.
4. Olivetto I, Girolami F, Ackerman MJ, et al. Myofibrin protein gene mutation screening and outcome of patients with hypertrophic cardiomyopathy. *Mayo Clin Proc* 2008;83:630–8.
5. Richard PP, Charron P, Carrier L, et al., for the EUROGENE Heart Failure Project. Hypertrophic cardiomyopathy: distribution of disease genes, spectrum of mutations, and implications for a molecular diagnosis strategy. *Circulation* 2003;107:2227–32.
6. Erdmann J, Daehmlow S, Wischke S, et al. Mutational spectrum in a large cohort of unrelated consecutive patients with hypertrophic cardiomyopathy. *Clin Genet* 2000;64:339–49.
7. Van Driest SL, Ellsworth EG, Ommen SR, et al. Prevalence and spectrum of thin filament mutations in an outpatient referral population with hypertrophic cardiomyopathy. *Circulation* 2003;108:445–51.

8. Van Driest SL, Vasile VC, Ommen SR, et al. Myosin binding protein C mutations and compound heterozygosity in hypertrophic cardiomyopathy. *J Am Coll Cardiol* 2004;44:1903–10.
9. Alpert NR, Mohiddin SA, Tripodi D, et al. Molecular and phenotypic effects of heterozygous, homozygous, and compound heterozygote myosin heavy-chain mutations. *Am J Physiol Heart Circ Physiol* 2005;288:H1097–102.
10. Tsoutsman T, Kelly M, Ng DC, et al. Severe heart failure and early mortality in a double-mutation mouse model of familial hypertrophic cardiomyopathy. *Circulation* 2008;117:1820–31.
11. Ingles J, Doolan A, Chiu C, Seidman J, Seidman C, Semsarian C. Compound and double mutations in patients with hypertrophic cardiomyopathy: implications for genetic testing and counselling. *J Med Genet* 2005;42:e59.
12. Kelly M, Semsarian C. Multiple mutations in genetic cardiovascular disease: a marker of disease severity. *Circ Cardiovasc Genet* 2009;2:182–90.
13. Maron MS, Olivetto I, Betocchi S, et al. Effect of left ventricular outflow tract obstruction on clinical outcome in hypertrophic cardiomyopathy. *N Engl J Med* 2003;348:295–303.
14. Clinical Molecular Genetics Society (CMGS). Practice guidelines for the interpretation and reporting of unclassified variants (UVs) in clinical molecular genetics. Available at: <http://www.cmgs.org/BPGs/pdfs%20current%20bpgs/UV%20GUIDELINES%20ratified.pdf>. Accessed October 28, 2009.
15. Maron BJ, Spirito P, Shen WK, et al. Implantable cardioverter-defibrillators and prevention of sudden cardiac death in hypertrophic cardiomyopathy. *JAMA* 2007;298:405–12.
16. Yacoub MH, Olivetto I, Cecchi F. 'End-stage' hypertrophic cardiomyopathy: from mystery to model. *Nat Clin Pract Cardiovasc Med* 2007;4:232–3.
17. Biagini E, Coccolo F, Ferlito M, et al. Dilated-hypokinetic evolution of hypertrophic cardiomyopathy: prevalence, incidence, risk factors, and prognostic implications in pediatric and adult patients. *J Am Coll Cardiol* 2005;46:1543–50.
18. Harris KM, Spirito P, Maron MS, et al. Prevalence, clinical profile, and significance of left ventricular remodeling in the end-stage phase of hypertrophic cardiomyopathy. *Circulation* 2006;114:216–25.
19. Hilfiker-Kleiner D, Knöll D. Disease-modifying mutations in familial hypertrophic cardiomyopathy: complexity from simplicity. *Circulation* 2008;117:1775–7.
20. Ho CY, Seidman CE. A contemporary approach to hypertrophic cardiomyopathy. *Circulation* 2006;113:e858–62.
21. Marian AJ. Genetic determinants of cardiac hypertrophy. *Curr Opin Cardiol* 2008;23:199–205.
22. Adabag AS, Maron BJ, Appelbaum E, et al. Occurrence and frequency of arrhythmias in hypertrophic cardiomyopathy in relation to delayed enhancement on cardiovascular magnetic resonance. *J Am Coll Cardiol* 2008;51:1369–74.
23. Belus A, Piroddi N, Scellini B, et al. The familial hypertrophic cardiomyopathy-associated myosin mutation R403Q accelerates tension generation and relaxation of human cardiac myofibrils. *J Physiol* 2008;586:3639–44.
24. Olivetto I, Cecchi F, Gistri R, et al. Relevance of coronary microvascular flow impairment to long-term remodeling and systolic dysfunction in hypertrophic cardiomyopathy. *J Am Coll Cardiol* 2006;47:1043–8.
25. Sotgia B, Sciagrà R, Olivetto I, et al. Spatial relationship between coronary microvascular dysfunction and delayed contrast enhancement in patients with hypertrophic cardiomyopathy. *J Nucl Med* 2008;49:1090–6.
26. Keren A, Syrris P, McKenna WJ. Hypertrophic cardiomyopathy: the genetic determinants of clinical disease expression. *Nat Clin Pract Cardiovasc Med* 2008;5:747.
27. Ho CY, Lever HM, DeSanctis R, Farver CF, Seidman JG, Seidman CE. Homozygous mutation in cardiac troponin T: implications for hypertrophic cardiomyopathy. *Circulation* 2000;102:1950–5.

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