ORIGINAL ARTICLE

Myofilament Protein Gene Mutation Screening and Outcome of Patients With Hypertrophic Cardiomyopathy

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OBJECTIVE: **To determine the influence of a positive genetic test for hypertrophic cardiomyopathy (HCM) on clinical outcome.**

PATIENTS AND METHODS: **A cohort of 203 unrelated patients with HCM (mean ± SD age, 50±18 years) was enrolled from January 1, 2002, through December 31, 2003. They were followed up for a mean ± SD time of 4.0±1.7 years after genetic testing of the 8 HCM-susceptibility genes that encode key sarcomeric/myofilament proteins. The clinical phenotype of those with a positive genetic test (myofilament-positive HCM) was compared with those with a negative genetic test (myofilament-negative HCM).**

RESULTS: **In this cohort of 203 patients, 87 mutations were identified in 126 patients (myofilament-positive HCM, 62%); the remaining 77 patients (38%) were myofilament-negative. Despite similar baseline features, patients with myofilament-positive HCM showed increased risk of the combined end points of cardiovascular death, nonfatal stroke, or progression to New York Heart Association class III or IV compared with the patients with myofilament-negative HCM (25% vs 7%, respectively; independent hazard ratio, 4.27; *P*=.008). These end points occurred at any age among patients with myofilament-positive HCM (range, 14-86 years), but only in those aged 65 years and older among patients with myofilament-negative HCM. Moreover, patients with myofilament-positive HCM showed greater probability of severe left ventricular systolic and diastolic dysfunction, defined as an ejection fraction of less than 50% and a restrictive filling pattern (*P*=.02 and *P*<.02, respectively, vs myofilament-negative HCM).**

CONCLUSION: **Screening for sarcomere protein gene mutations in HCM identifies a broad subgroup of patients with increased propensity toward long-term impairment of left ventricular function and adverse outcome, irrespective of the myofilament (thick, intermediate, or thin) involved.**

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CI = confidence interval; DHPLC = denaturing high-performance liquid chromatography; HCM = hypertrophic cardiomyopathy; HR = hazard ratio; LV = left ventricular; NYHA = New York Heart Association

Hypertrophic cardiomyopathy (HCM) is the most common genetic cardiac disease, characterized by heterogeneous morphologic expression and clinical course.[1-3] Mutations in genes coding for myofilament contractile proteins of the cardiac sarcomere represent the most common genetic subtype of HCM, with a 30% to 65% prevalence in various cohort studies.[4-8] Thus, HCM has been repeatedly defined as a disease of the sarcomere.[1,9] Early reports fostered the notion that genotyping of patients with HCM might prove useful for risk stratification, with particular regard to sudden cardiac death.[10-12] However, subsequent studies have failed to establish meaningful relationships among individual sarcomere gene mutations, phenotype, and outcome in HCM cohorts.[1-3,12,13]

Indeed, virtually all studies aimed at identifying and emphasizing differences among individual myofilament genes associated with HCM have in fact shown vast overlap of clinical and pathophysiologic correlates among subgroups.[1-3,10-15] Remarkably, no attempts have been made to collectively characterize the clinical profile of patients with myofilament-positive HCM (ie, patients with identifiable sarcomeric mutations) vs myofilament-negative HCM. To address this gap in the data, our study prospectively assessed the clinical features and outcome of unrelated patients from a large, consecutive HCM cohort after comprehensive mutational screening of the 8 myofilament protein genes that currently form the basis of commercially available genetic tests for HCM.

For editorial comment, see page 626

PATIENTS AND METHODS

The study included 203 unrelated index patients with a confirmed clinical diagnosis of HCM, consecutively enrolled at the Azienda Ospedaliera-Universitaria Careggi, in Florence, Italy, and Ospedale San Camillo, in Rome, Italy, since the beginning of a systematic screening

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TABLE 1. **Demographic and Clinical Features of 203 Study Patients With HCM[a,b]**

	Overall study cohort (n=203)	Myofilament		*P* value
		Positive (n=126)	Negative (n=77)	
Female	73 (36)	45 (36)	28 (36)	>.99
Age at initial HCM diagnosis (y)	43±18	41±18	46±17	.06
Age at DNA collection (y)	50±18	49±18	53±17	.13
Age at final evaluation (y)	54±18	53±18	57±17	.14
Family history of HCM	57 (28)	44 (35)	13 (17)	<.01
Family history of sudden death	29 (14)	23 (18)	6 (8)	<.05
Follow-up from initial HCM diagnosis (y)	11.3±8.6	11.6±8.3	10.9±8.9	.50
Follow-up from DNA collection (y)	4.0±1.7	3.9±1.7	4.2±1.6	.70
Clinical and echocardiographic features at DNA collection				
NYHA class	1.8±0.8	1.8±0.8	1.9±0.7	.50
Syncope	27 (13)	16 (13)	11 (14)	.80
Atrial fibrillation[c]	62 (31)	43 (34)	19 (25)	.20
Paroxysmal	27 (13)	15 (12)	12 (16)	.52
Chronic	35 (17)	28 (22)	7 (9)	.02
Left atrium (mm)	46±9	47±10	45±8	.30
Resting LV outflow obstruction (≥30 mm Hg)	60 (30)	32 (25)	28 (36)	.10
Maximum LV thickness (mm)	23±6	24±6	23±5	.15
Maximum LV thickness ≥30 mm	32 (16)	23 (18)	9 (12)	.24
LV end-diastolic dimension (mm)	45±6	45±6	45±6	.98
LV end-systolic dimension (mm)	27±7	27±7	27±6	.70
LV ejection fraction (%)	62±9	61±9	62±7	.80
Interventions during follow-up				
Alcohol septal ablation	23 (11)	14 (11)	9 (12)	.80
Surgical septal myectomy	15 (7)	7 (6)	8 (10)	.30
Other cardiac surgery (mitral valve replacement/valvuloplasty, CABG)	12 (6)	11 (9)	1 (1)	.03
Implantable cardioverter-defibrillator	28 (14)	21 (17)	7 (9)	.14
Outcome at 5 y[d]				
Unfavorable	36 (16)	31 (22)	5 (8)	.002[e]
Progression to NYHA class III or IV[f]	20 (12)	18 (16)	2 (3)	
Nonfatal ischemic stroke	2 (1)	2 (2)	0 (0)	
CV death	14 (7)	11 (8)	3 (5)	
Sudden-unexpected	2 (1)	2 (1)	0 (0)	
Heart failure–related	8 (4)	6 (5)	2 (4)	
Other (postoperative, MI, stroke)	4 (2)	3 (3)	1 (1)	

[a] CABG = coronary artery bypass graft; CV= cardiovascular; HCM = hypertrophic cardiomyopathy; LV = left ventricular; MI = myocardial infarction; NYHA = New York Heart Association.

[b] Categorical data are presented as number of patients (percentage) and continuous data as mean ± SD, unless otherwise indicated. All characteristics presented in the Table have been assessed in each of the 203 study patients.

[c] Warfarin treatment was systematically given to patients with paroxysmal or chronic AF, unless a major contraindication was present. With the exception of 2 patients, all patients with chronic AF were receiving long-term warfarin treatment.

[d] Number in parentheses indicates 5-year Kaplan-Meier cumulative event rate estimates, rather than absolute percentages, to account for varying duration of follow-up.

[e] Assessed by survival analysis.

[f] Excluding patients already in NYHA class III or IV at the time of DNA collection.

program for myofilament gene mutations.[13] Patients were enrolled from January 1, 2002, through December 31, 2003. The study patients had already been diagnosed as having HCM and were followed up for a median of 4.1 years before genetic screening became available. Of note, 66 patients (33%) had been followed up for more than 10 years before enrollment in the current study. All patients were white and of Italian origin. Mean ± SD age at enrollment was 50±18 years; 73 patients (36%) were female. Maximum left ventricular (LV) thickness ± SD was 23±6 mm, and 59 patients (29%) had resting LV outflow tract obstruction (peak gradient, >30 mm Hg) (Table 1). Diagnosis of HCM was based on 2-dimensional echocardiographic identification of a hypertrophied, nondilated left ventricle, in the absence of another cardiac or systemic disease capable of producing the magnitude of ventricular hypertrophy.[1,2] A family history of HCM was actively investigated in all patients in the course of routine

pre–genetic test counseling by a geneticist and a clinical cardiologist. Available medical records of deceased relatives were also systematically reviewed.

Echocardiography

We performed echocardiographic studies with commercially available instruments. We assessed LV hypertrophy with 2-dimensional echocardiography, identifying the site of maximal wall thickness. We considered a peak instantaneous outflow gradient of 30 mm Hg or more, estimated with continuous wave Doppler echocardiography under basal conditions, to be indicative of LV outflow obstruction.[1] We measured LV ejection fraction using the biplane Simpson rule and assessed LV filling pattern by pulse-wave Doppler study at the mitral tip level.

Mutational Analysis

DNA Extraction and Polymerase Chain Reaction. After informed consent was obtained, genomic DNA was extracted from peripheral blood according to QIAamp DNA Blood BioRobot MDx kit (QIAGEN GmbH, Hilden, Germany). In vitro amplification of all candidate exons was performed by polymerase chain reaction using primers described previously.[13,16,17]

Identification of Mutations. Patients were screened for mutations in the protein-coding exons and splice sites of 8 candidate myofilament genes encoding myosin-binding protein C (*MYBPC3*), thick-filament proteins (β-myosin heavy chain [*MYH7*] and the regulatory and essential light chains [*MYL2* and *MYL3*]), and thin-filament proteins (troponin T type 2 [*TNNT2*], troponin I type 3 [*TNNI3*], α-tropomyosin [*TPM1*], and α-actin [*ACTC*]). These HCM-susceptibility genes are the basis of commercially available genetic tests for HCM (for a comprehensive list of mutations and polymorphisms in sarcomere protein genes associated with HCM, see http://genetics.med.harvard.edu/~seidman/cg3; for genetic tests, see http://www.correlagen.com). Sequence variations were detected by denaturing high-performance liquid chromatography (DHPLC) using the WAVE DNA Fragment Analysis System (Transgenomics, San Jose, CA). The conditions for DHPLC were developed on the basis of exon-specific melting profiles predicted by NAVIGATOR software (Transgenomic, Omaha, NE).[18]

Abnormal DHPLC elution profiles were sequenced on automated dye terminator cycle sequencing using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, KS). Every mutation identified was confirmed by a new polymerase chain reaction, and, when possible, by restriction enzyme digestion. Novel mutations were considered as disease-causing only if they were absent in 300 unrelated chromosomes from adult, ethnically matched healthy control participants and they produced a change in a highly conserved residue among species and isoforms. Cosegregation of the mutation with the disease was sought in the probands' families. All patients and relatives received pretest and post-test genetic counseling.

Follow-up Protocol

The follow-up protocol and chosen end points for the study cohort were the same used for the overall HCM population, as previously described.[19] Patients were followed up at yearly intervals or more often if required, with clinical and echocardiographic examination, 12-lead electrocardiography, and 24- to 48-hour ambulatory electrocardiography.

Study End Points

The first clinical end point was cardiovascular death, including death related to heart failure, myocardial infarction, or stroke; sudden unexpected death, including resuscitated cardiac arrest; and postoperative death after cardiac surgery. This end point also proved equivalent to all-cause mortality, as no noncardiovascular deaths occurred. The second clinical end point was unfavorable outcome, a combined end point including cardiovascular death, nonfatal stroke, or progression to severe functional limitation (New York Heart Association [NYHA] class III or IV) at final evaluation.

Echocardiographic end points used in this study were systolic dysfunction, defined as an LV ejection fraction less than 50% at final evaluation; and a restrictive LV filling pattern, considered evidence of severe diastolic dysfunction and defined by a mitral ratio of peak early to late diastolic filling velocity (E/A ratio) of 2 or more in conjunction with deceleration time of 150 ms or less or, in patients with atrial fibrillation, by a transmitral deceleration time of less than 120 ms.[20,21]

Statistical Analyses

Unpaired *t* test was used for the comparison of normally distributed data. The χ^2 or Fisher exact test was used to compare noncontinuous variables expressed as proportions. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated using univariate and multivariate Cox proportional hazard regression models. Multivariate analysis was performed with a stepwise forward regression model, in which each variable with $P<.05$ (based on univariate analysis) was entered into the model. Survival curves were constructed according to the Kaplan-Meier method, and comparisons were performed using the log-rank test. Time of genetic testing was considered as time 0 for clinical end points. However, to assess the lifelong occurrence of LV dysfunction with respect to myofilament

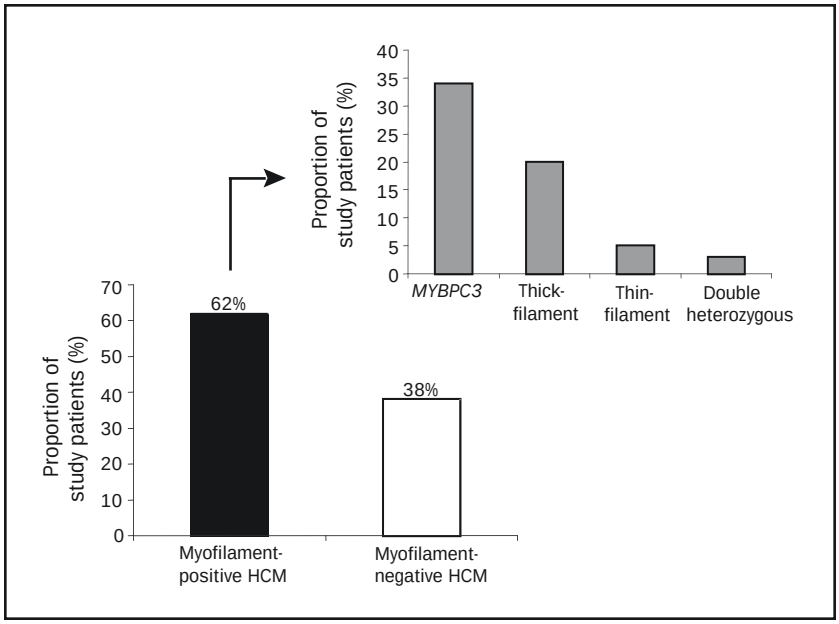

FIGURE 1. Prevalence and distribution of sarcomeric myofilament protein gene mutations among the 203 study patients with hypertrophic cardiomyopathy (HCM). All percentages are calculated with regard to the entire patient cohort.

mutation status, age was used as the time scale in assessing echocardiographic end points.[22] Patients with multiple mutations counted as single individuals in all analyses. *P* values are 2-sided and considered significant when <.05. Calculations were performed using SPSS 12.0 software (SPSS, Chicago, IL).

RESULTS

Comprehensive Mutational Analysis

In 126 (62%) of the 203 study patients, we identified 87 distinct mutations, of which 51 were classified as novel (Figure 1, Table 2). These 126 patients were considered to have myofilament-positive HCM. The remaining 77 patients (38%) had no discernible myofilament mutations and were considered to have myofilament-negative HCM. The most frequent disease-associated gene was *MYBPC3* (n=68; 34%; including 9 patients with double mutations in this gene). The *MYBPC3* E258K mutation, which was found in 27 index patients, suggested a founder effect and was associated with heterogeneous cardiac morphology and clinical presentation. Among the other patients, 41 (20%) had mutations in thick-filament protein genes (34 in *MYH7* and 7 in *MYL2*) and 11 (5%) in thin-filament protein genes (7 in *TNNT2*, 2 in *TPM1*, 1 in *TNNI3*, and 1 in *ACTC*). Six patients (3%) were double heterozygous (ie, carried mutations in 2 different genes; Table 2).

Morphologic features of patients with myofilament-positive or myofilament-negative HCM were indistinguishable on the basis of clinical, instrumental, and postoperative histologic findings. Age at the time of genetic testing, sex, NYHA functional class, maximum LV wall thickness, and prevalence of resting LV outflow tract obstruction were comparable in patients with myofilament-positive and myofilament-negative HCM. The overall prevalence of atrial fibrillation was not significantly different in the 2 groups, although the chronic form of the arrhythmia was more prevalent in the myofilament-positive patients. Follow-up duration and referral rates for septal myectomy, alcohol septal ablation, or implantation of a cardioverter-defibrillator were similar. However, patients with myofilament-positive HCM more often had family history of HCM and sudden cardiac death (Table 1).

Clinical Course and Outcome

Median follow-up after genetic screening was 4.5 years, for a total of 817 patient-years. During follow-up, 14 patients (7%) died from cardiovascular causes (2 suddenly, 8 owing to heart failure, and 4 owing to other causes, including postoperative complications of surgical myectomy [n=2], myocardial infarction, and stroke). In addition, 20 patients (10%) progressed to NYHA functional class III or IV, and 2 had a cardioembolic stroke. Overall, an unfavorable outcome occurred in 36 patients (18%); 1-, 3-, and 5-year Kaplan-Meier cumulative event rate estimates

TABLE 2. **Myofilament Gene Mutations Associated With Hypertrophic Cardiomyopathy in 203 Unrelated Index Patients**[a,b,c]

Exon/ intron	Nucleotide change	Mutation	Mutation type	No. of patients with mutation
		MYBPC3 gene		
E4	GAG>GAC	E165D[d]	Missense[e]	1
IVS5	a>c	IVS5-2a>c	Splice mutation	1
E5	AGC>AGG	S212R[d]	Missense	1
E6	GAG>GAC	E240D[d]	Missense	1
E6	GAG>AAG	E258K	Splice or missense[e]	26
E7	CGC>CAG	R273H	Missense[e]	1
E7	g>a	IVS7+1g>a	Splice or missense[e]	1
E11	delTT	F305fs[d]	Frameshift/ter	1
IVS12	g>a	IVS12+1g>a[d]	Splice mutation	2
E12	GAG>AAG	E334K[d]	Missense	1
E12	TAC>TAG	Y340X[d]	Nonsense[e]	2
E13	delG	A392fs[d]	Frameshift/ter[e]	2
E15	GAG>AAG	E441K[d]	Missense	1
E16	CGG>TGG	R470W[d]	Missense	1
E17	GGG>AGG	G490R	Missense[e]	1
E17	GGG>AGG	G531R	Missense[e]	1
E17	CGG>CAG	R502Q	Missense[e]	2
E17	CGC>AGC	A522T[d]	Missense[e]	1
E17	TAT>TCT	Y525S[d]	Missense	1
IVS17	GAA>CAA	E542Q	Splice or missense[e]	3
IVS17	g>a	IVS17-2g>a[d]	Splice or missense	1
IVS18	c>t	IVS18+1c>t[d]	Frameshift/ter	1
E18	ATG>ACG	M555T[d]	Missense	1
E19	GAC>CAC	D610H[d]	Missense	1
E22	GCC>TCC	A693S[d]	Missense	1
E22	insA	T705fs	Frameshift/ter	1
E23	insT	V753fs	Frameshift/ter[e]	1
E23	delGTCATCG	V768fs[d]	Frameshift/ter[e]	1
E23	GAC>AAC	D770N	Splice or missense	1
E24	GTG>ATG	V771M	Missense[e]	1
E24	GAC>TAC	D786Y[d]	Missense	1
IVS24	a>g	IVS24-2a>g	Splice mutation[e]	2
E25	delAAG	K814fs	Frameshift/ter	1
E25	CGG>CAG	R820Q	Missense	1
E26	delGGAGCAGGAG	G897fs[d]	Frameshift/ter[e]	1
E26	delT	Y904fs[d]	Frameshift/ter	1
E26	CCA>ACA	P910T[d]	Missense	1
E27	CAA>TAA	Q969X	Nonsense	1
E30	insC	K1065fs	Frameshift/ter[e]	6
E31	insT	W1112fs[d]	Frameshift/ter	1
E31	insGGTT	G1146fs[d]	Frameshift/ter	1
E32	ACC>AAC	T1184N[d]	Missense	1
E32	CTG>CGG	L1187R[d]	Missense	1
E32	GGT>GTT	G1206V[d]	Missense	1
E32	GGT>GAT	G1206D[d]	Missense	1
E33	CGA>TGA	R1271X[d]	Nonsense	1
		MYH7 gene		
E8	GCC>TCC	A233S[d]	Missense	1
E10	AAA>AGA	K270R[d]	Missense[e]	1
E13	GGG>GAG	G398E[d]	Missense	1
E13	CGG>CAG	R403Q	Missense[e]	2
E14	CGC>TGC	R442C[d]	Missense[e]	1
E16	CTC>TTC	L601F[d]	Missense	1
E16	GTG>ATG	V606M	Missense[e]	1
E16	CGC>CAC	R663H	Missense[e]	2
E16	CGC>TGC	R663C	Missense	1
E17	AGG>GGG	R652G	Missense[e]	1
E19	CGC>TGC	R694C[d]	Missense[e]	2
E20	CGC>TGC	R723C	Missense[e]	1
E21	AGC>AGA	S782R[d]	Missense	1
E21	GCC>ACC	A797T	Missense	2
E22	CGC>CCC	R858P[d]	Missense	1
E22	AAG>AGG	K865R[d]	Missense	1
E22	CGC>CAC	R869H	Missense[e]	10
E23	ATC>ATG	I909M[d]	Missense[e]	1
E23	GAG>AAG	E927K[d]	Missense	1
E23	GAG>AAG	E965K[d]	Missense	1
E23	GAG>CAG	E930Q[d]	Missense	2
E25	CGC>TGC	R1045C[d]	Missense	1
E30	ACG>ATG	T1351M[d]	Missense[e]	1
E30	ACG>ATG	T1377M	Missense	1
E37	CTG>ATG	L1769M[d]	Missense	1
		MYL2 gene		
E2	ATG>CTG	M20L[d]	Missense[e]	3
E4	CGA>CAA	R58Q	Missense	2
E6	GAG>GCG	E134A[d]	Missense[e]	1
E7	GGA>AGA	E162R[d]	Missense	1
		TNNT2 gene		
E8	AAG>CAG	K66Q[d]	Missense[e]	1
E10	CGG>CAG	R92Q	Missense	1
E10	CGG>TGG	R92W	Missense	1
E10	TTT>TTG	F110L	Missense[e]	1
E11	CGC/TGC	R130C	Missense[e]	1
E12	delGAG	E160fs	Frameshift/ter	1
E17	CGC>TGC	R278C	Missense	2
		TPM1 gene		
E9	ATG>ACG	M281T	Missense[e]	1
E9	ATG>GTG	M284V[d]	Missense	1
		ACTC gene		
E5	TCT>TTT	S273F[d]	Missense	1
		TNNI3 gene		
E5	delC	A86fs	Frameshift/ter	1

[a] del = deletion; E = exon; fs = frameshift; ins = insertion; IVS = intervening sequences; ter = stop codon.

[b] Of the 87 mutations identified in our study, 51 were novel. Of these 51, most (33) were confirmed by cosegregation. The remaining 18, for which family studies could not be performed, were considered as disease-causing because they were absent in 300 unrelated chromosomes from adult, ethnically matched healthy controls and produced a change in a highly conserved residue among species and isoforms. For a more complete description of methodology, see reference 18.

[c] Nine patients carried a double *MYBPC3* mutation and were included among patients with *MYBPC3*-associated disease for survival analyses (A522T and V771M; E334K and R470W; IVS18+1c>t and A392fs; R502Q and G490R; T1184N and L1187R; G1206V and E240D; A693S and D786Y; E258K and P910T; E258K and E441K). Six additional patients were double heterozygous, ie, carried mutations in 2 different sarcomere genes (insC1065-*MYBPC3* and *MYH7* R869H; *MYBPC3* E258K and *MYH7* K865R; *MYBPC3* E258K and *MYH7* R694C; *MYBPC3* E165D and *MYH7* R273C; *MYBPC3* E542Q and *TNNT2* K66Q; *TNNI3* A86fs and *MYH7* R869H).

[d] Novel mutations.

[e] Tested for cosegregation in affected family members.

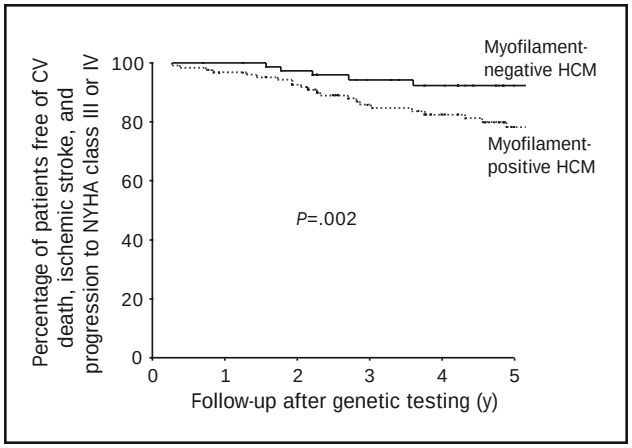

FIGURE 2. Relation of genetic status to the probability of cardiovascular (CV) death, nonfatal ischemic stroke, or progression to severe heart failure symptoms (New York Health Association [NYHA] functional classes III or IV) after genetic testing. HCM = hypertrophic cardiomyopathy.

for the whole cohort were 2%, 11%, and 16%, respectively (Table 1).

Among patients with myofilament-positive HCM, 1-, 3-, and 5-year rates for unfavorable outcome were 3%, 14%, and 22%, respectively. The combined end point occurred at ages ranging from 14 to 86 years (mean ± SD, 61±18 years; 7 of the 31 patients were younger than 50 years). There was no difference in outcome based on the affected myofilament (*MYBPC3*, thick or thin; overall, *P*=.45). Among patients with myofilament-negative HCM, 1-, 3-, and 5-year rates for unfavorable outcome were 0%, 6%, and 8%, respectively, and the end point occurred at ages ranging from 65 to 84 years (mean ± SD, 77±8 years). Difference in outcome between patients with myofilament-positive vs myofilament-negative HCM was highly significant (*P*=.002; Figure 2) and remained so even after adjusting for the potential *MYBPC3* founder effect by including only the first enrolled patient with the E258K mutation in the analysis (*P*=.003). Adjustment for other shared mutations did not change the final result (eg, for *MYH7* R869H; *P*=.005). In a multivariate model, myofilament-positive HCM was associated with unfavorable outcome; the association was independent of age, NYHA functional class, resting LV outflow obstruction, and atrial fibrillation (HR, 4.27; *P*=.008, Table 3).

TABLE 3. **Relation to Outcome of Clinical, Demographic, and Genetic Variables Evaluated at Time of Genetic Testing**[a]

	Hazard ratio (95% CI)	*P* value
Myofilament-positive HCM	4.27 (1.46-12.48)	.008
Age (per year)	1.03 (1.01-1.06)	.017
LV outflow obstruction (≥30 mm Hg at rest)	1.33 (0.66-2.67)	.43
Atrial fibrillation (paroxysmal or chronic)	1.67 (0.74-3.77)	.22

[a] Multivariate Cox proportional-hazards analysis was used in our evaluation. Variables excluded from the multivariate model (*P*>.05 at univariate analysis) were sex, left atrium diameter, left ventricular (LV) end-diastolic diameter, LV end-systolic diameter, LV ejection fraction, maximum LV wall thickness, syncope, and family history of sudden death. CI = confidence interval; HCM = hypertrophic cardiomyopathy.

With regard to the specific end point of cardiovascular death, the difference between patients with myofilament-positive and myofilament-negative HCM was not statistically significant (5-year cumulative event rate, 8% vs 5%; *P*=.19). Nevertheless, myofilament-positive HCM accounted for 11 of the 14 cardiovascular deaths, including both sudden deaths and 6 of 8 due to heart failure (Table 1). All 3 cardioembolic strokes (1 fatal and 2 nonfatal) occurred in the myofilament-positive group.

Prevalence of LV Dysfunction

At final echocardiographic evaluation, 50 patients (25%) had severe LV impairment, including 22 with systolic dysfunction (ie, end-stage phase) and 28 with restrictive filling pattern (Table 1). Cumulative rates of LV impairment at 1, 3, and 5 years were 4%, 7% and 17%, respectively. Of the 22 patients with systolic dysfunction, 18 (82%) had myofilament-positive HCM owing to mutations in *MYBPC3* (n=13), *MYH7* (n=2), *MYL2* (n=1), *TPM1* (n=1), and in a *MYBPC3* and *MYH7* double heterozygote. Of note, systolic impairment was common among the 27 probands with the *MYBPC3* E258K mutation (n=9; 33%). Conversely, only 4 patients (18%) with severe systolic dysfunction had myofilament-negative HCM. Patients with severe LV impairment included 22 (61%) of the 36 patients with an unfavorable outcome.

Patients with myofilament-positive HCM showed greater probability of severe LV systolic and/or diastolic dysfunction than those with myofilament-negative HCM (HR, 2.1; 95% CI, 1.1-4.0; *P*=.02; Figure 3). No significant difference was found on the basis of the affected myofilament (*MYBPC3*, thick or thin); although double heterozygous patients appeared to be at higher risk (Figure 3). These results were unchanged after adjustment for the potential *MYBPC3* E258K founder effect (HR for development of systolic and/or diastolic dysfunction, 2.1; 95% CI, 1.1-4.2; *P*=.02).

DISCUSSION

Our study shows that the identification of 1 or more cardiac myofilament gene mutations is associated with increased risk of LV dysfunction and adverse outcome due to heart failure in patients with HCM. In our cohort, myofilament-positive HCM was characterized by an almost 30% prevalence of severe systolic and/or diastolic LV dysfunction,

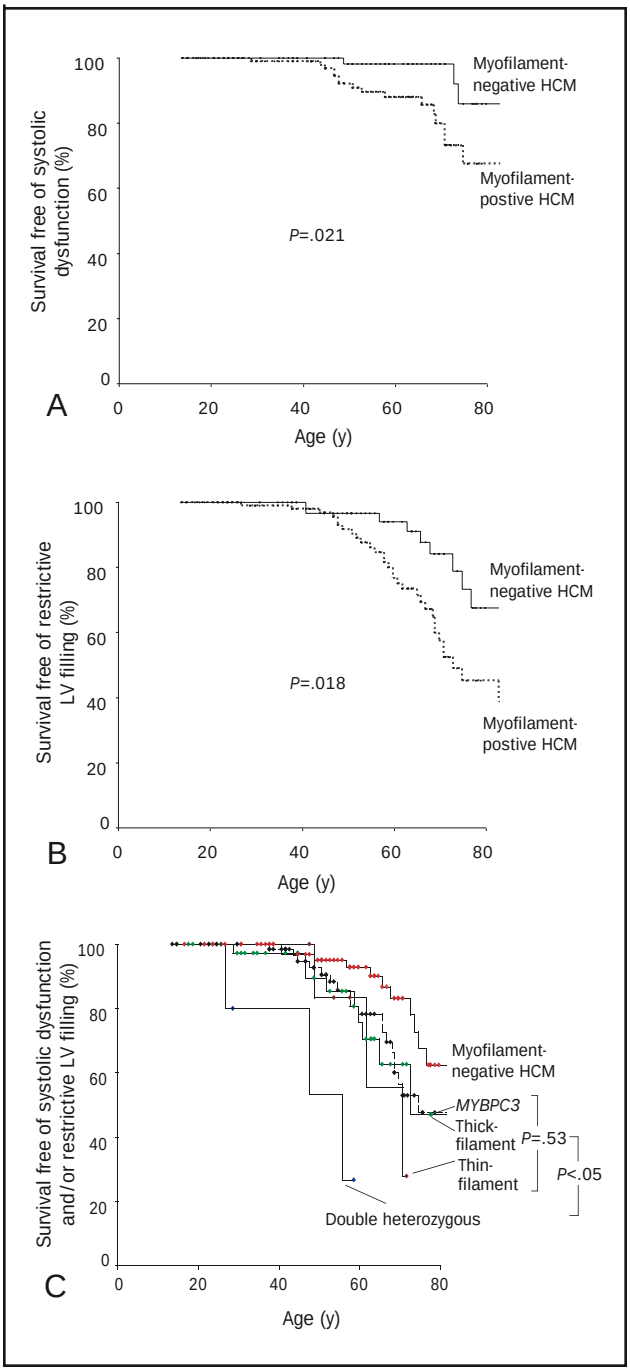

FIGURE 3. Cumulative occurrence of left ventricular (LV) dysfunction in relation to individual age and myofilament genetic status. Patients were censored at the age of first documentation of LV dysfunction, as defined for each panel. A, Survival free of systolic dysfunction, defined as LV ejection fraction <50%; B, Survival free of restrictive LV filling pattern, defined as transmitral deceleration time <120 ms or a mitral ratio of peak early to late diastolic filling velocity (E/A ratio) of ≥2 in conjunction with a deceleration time of ≤150 ms; C, Survival free of both systolic dysfunction and restrictive LV filling (whichever occurred first).

which was increasingly common after the fourth decade of life and occurred at a similar rate for each of the 3 major myofilament subtypes involved. By comparison, myofilament-negative HCM was associated with later onset and an approximately 50% lower risk of LV impairment. In an average of 4 years after genetic testing, the difference in prevalence of LV dysfunction in patients with myofilament-positive vs myofilament-negative HCM was reflected in a more severe outcome in the former, mostly due to heart failure. In the myofilament-positive HCM group, 25% of patients experienced an unfavorable outcome (defined as the combination of cardiovascular death, nonfatal stroke, or progression to NYHA classes III or IV), occurring in all age groups including young adults and adolescents. Conversely, only 7% of patients with myofilament-negative HCM reached the same end point, and all of these patients were 65 years or older. Such difference in outcome, which was evident despite virtually indistinguishable clinical features and risk profile at the time of genetic testing, proved highly significant (*P*=.002). In a multivariate model, which included established predictors of risk for HCM such as NYHA functional class, resting LV outflow obstruction, and atrial fibrillation, the presence of any myofilament gene mutation was associated with a more than 4-fold independent increase in risk of an unfavorable outcome when compared with patients with HCM who had negative results on genetic testing.

Our findings suggest that myofilament-positive HCM is frequently characterized by progressive impairment of LV diastolic and systolic function,[23] leading to clinical deterioration and heart-failure–related complications, including atrial fibrillation and stroke, in a sizeable proportion of patients.[19,22,24] Specifically, most (82%) of the 22 patients with LV systolic dysfunction at last evaluation (ie, the end-stage phase) had myofilament-positive HCM, caused by mutations in 4 different genes (*MYBPC3*, *MYH7*, *MYL2*, *TPM1)*. Systolic dysfunction was particularly common in a subset of 27 unrelated index patients sharing the *MYBPC3* E258K mutation, likely because of a founder effect. Such observations are in close agreement with the recent report by Kubo et al,[22] showing progressive LV remodeling and adverse outcome in patients with a founder-effect, frameshift/deletion mutation in *MYBPC3*. Thus, although the end-stage phase does not appear to be confined to any specific genetic substrate within the sarcomere, a preferential role in promoting systolic dysfunction may be hypothesized for *MYBPC3*-encoded myosin-binding protein C.[22,25]

Overall, our observations support a clinical role for systematic genetic testing in the index patient with HCM and have potential implications for management. Specifically, the greater risk of heart failure–related compli-

cations may warrant closer follow-up in patients with myofilament-positive HCM to promptly detect LV dysfunction; manage treatable features, such as atrial fibrillation, outflow tract obstruction, and comorbidity; and anticipate changes in clinical course.[24,26] In contrast, the identification of patients with myofilament-negative HCM is likely to lead to more in-depth clinical and genetic work-up exploring rare conditions causing (or mimicking) HCM.[2,27,28]

The processes leading from single-gene mutations to development of clinically recognized HCM, and from phenotypic expression of a generally hyperdynamic LV to functional impairment, are still largely unknown.[23] Hypotheses that mutant sarcomere myofilament proteins depress contractile function, leading to activation of the signaling pathways for compensatory hypertrophy,[29] are inconsistent with recent evidence of gain of function of HCM-related mutant myosins.[30] Pilot mechanical experiments in human cardiac myofibrils also indicate that the kinetics of specific steps of the force-generating adenosine triphosphatase reaction are faster in the sarcomeres of patients with HCM who carry *MYBPC3* or *MYH7* mutations, leading to increased energy consumption, than in sarcomeres of control patients.[31]

From a pathophysiologic standpoint, marked impairment in whole-heart energetics associated with mutations of several sarcomeric proteins in patients with HCM[32] and in animal models[33,34] suggest that increased energy cost of cardiac function may be a common feature of several HCM-susceptibility genes.[35] Our findings support the idea that similar maladaptive mechanisms, triggered by inefficient sarcomere energy use, may occur in myofilament-positive HCM, irrespective of the gene involved, leading progressively to LV impairment.[23] As a consequence, the site of individual sarcomere gene mutations would not qualitatively affect the pathophysiologic mechanisms involved so much as it would determine the process quantitatively.[36] This concept is further supported by the finding that double heterozygous patients with HCM showed greater and earlier LV impairment, likely reflecting a more profound derangement of sarcomeric protein function.[37]

We are aware that to consider all patients with myofilament-negative HCM as a single group is an unavoidable oversimplification of our current study and that further research in this area is needed. Myofilament-negative HCM is thought to be a composite entity, probably comprising a multitude of rare, heterogeneous, and yet-to-be-identified HCM-susceptibility genes, including rare high-risk subsets.[2,27] We also acknowledge the likelihood that a proportion of patients currently classified as myofilament-negative may nevertheless have HCM-susceptibility mutations in novel myofilament-encoding genes or in unexplored regions (eg, promoters, introns) of the known HCM-associated myofilament genes. In clinical practice, each patient with such mutations deserves in-depth individual assessment until the entire molecular and clinical spectrum of HCM is unraveled.[23,38]

The quest for novel disease-causing genes in HCM is ongoing. Currently, efforts are moving along 2 main pathways. The first addresses genes coding for Z-disk proteins, which are essential for the structural organization of the cardiac sarcomere and the cardiomyocyte's stretch sensor. These include titin (*TTN*), muscle LIM protein (*CSRP3*), telethonin (*TCAP*), and myozenin 2 (*MYOZ2*).[38] Recently, an HCM case associated with a mutation in the vinculin gene was reported.[39] Vinculin is a cytoskeletal protein localized to intercalated disks and functions as an actin-interacting protein that anchors the thin filaments to the intercalated disks. The second pathway follows the description of mutations in the γ2 subunit of adenosine monophosphate–dependent protein kinase (*PRKAG2*) and in the lysosomal-associated membrane protein 2 (*LAMP2*), which cause unexplained hypertrophy resembling HCM.[27,28] These findings suggest a link between such entities and storage syndromes such as Fabry disease.[38]

The rate of unfavorable outcome in our cohort of patients with HCM was relatively high (16% at 5 years), compared with prior reports that were largely based on patients with newly diagnosed disease.[19,26] However, patients in the current study already had a substantial history of disease at the time of enrollment, including approximately one-third (66; 33%) with a clinical diagnosis dating back more than 10 years. We think such clinical background largely accounts for the seemingly higher rate of disease-related complications.

CONCLUSION

Hypertrophic cardiomyopathy due to myofilament gene mutations is characterized by adverse outcome and more frequent derangement of LV function compared with myofilament-negative disease, irrespective of the myofilament type involved. These findings support a clinical and prognostic role for genetic testing in patients with clinically diagnosed HCM.

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