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Microvascular Function Is Selectively Impaired in Patients With Hypertrophic Cardiomyopathy and Sarcomere Myofilament Gene Mutations

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Objectives   The purpose of this study was to assess myocardial blood flow (MBF) using positron emission tomography in patients with hypertrophic cardiomyopathy (HCM) according to genetic status.

Background   Coronary microvascular dysfunction is an important feature of HCM, associated with ventricular remodeling and heart failure. We recently demonstrated the increased prevalence of systolic dysfunction in patients with HCM with sarcomere myofilament gene mutations and postulated an association between genetic status and coronary microvascular dysfunction.

Methods   Maximum MBF (intravenous dipyridamole, 0.56 mg/kg; Dip-MBF) was measured using ^{13}N-labeled ammonia in 61 patients with HCM (age 38 ± 14 years), genotyped by automatic DNA sequencing of 8 myofilament-encoding genes (myosin-binding protein C, beta-myosin heavy chain, regulatory and essential light chains, troponin T, troponin I, troponin C, alpha-tropomyosin, and alpha-actin). In 35 patients, cardiac magnetic resonance imaging was performed.

Results   Fifty-three mutations were identified in 42 of the 61 patients (genotype positive; 69%). Despite similar clinical profiles, genotype-positive patients with HCM showed substantially lower Dip-MBF compared with that of genotype-negative patients (1.7 ± 0.6 ml/min/g vs. 2.4 ± 1.2 ml/min/g; $p < 0.02$). A Dip-MBF <1.5 ml/min/g had 81% positive predictive value for genotype-positive status and implied a 3.5-fold independent increase in likelihood of carrying myofilament gene mutations (hazard ratio: 3.52; 95% confidence interval: 1.05 to 11.7; $p = 0.04$). At cardiac magnetic resonance imaging, the prevalence of late gadolinium enhancement was greater in genotype-positive patients (22 of 23 [96%] compared with 8 of 12 [67%] genotype-negative patients; $p = 0.038$).

Conclusions   Patients with HCM with sarcomere myofilament mutations are characterized by more severe impairment of microvascular function and increased prevalence of myocardial fibrosis, compared with genotype-negative individuals. These findings suggest a direct link between sarcomere gene mutations and adverse remodeling of the microcirculation in HCM, accounting for the increased long-term prevalence of ventricular dysfunction and heart failure in genotype-positive patients.   (J Am Coll Cardiol 2011;58:839–48) © 2011 by the American College of Cardiology Foundation

Coronary microvascular dysfunction (CMD) is an important primary feature of hypertrophic cardiomyopathy (HCM), contributing to myocardial ischemia, left ventricular (LV) remodeling, and adverse outcomes (1–8). Whether the genetic substrate of HCM might influence remodeling of the coronary microcirculation, and thus impact the severity of CMD, remains to be determined (9).

We recently showed that patients with sarcomere myofilament gene mutations have a higher prevalence of systolic dysfunction and progression to the end-stage phase, compared with those with a negative genetic test (10). Of note, each of these clinical abnormalities is subtended by severe CMD in patients with HCM (4,6,11), a feature often evident many years before clinical or echocardiographic

Abbreviations and Acronyms

CMD = coronary microvascular dysfunction

Dip-MBF = myocardial blood flow following dipyridamole infusion

GLA = galactosidase

HCM = hypertrophic cardiomyopathy

LGE = late gadolinium enhancement

LV = left ventricle/ventricular

MYBPC3 = myosin-binding protein C

MYH7 = beta-myosin heavy chain

PET = positron emission tomography

VOI = volume of interest

evidence of disease progression (4,5). We therefore hypothesized that CMD might be more frequent and severe in HCM associated with proven sarcomere gene mutations, potentially accounting for differential outcomes based on genetic status (2). To address this issue, we used positron emission tomography (PET) to assess CMD in a cohort of patients who received comprehensive genetic testing for the principal HCM susceptibility genes. In a subset of patients, we also assessed the relationship between mutational status and prevalence of late gadolinium enhancement (LGE) assessed by cardiac magnetic resonance imaging (MRI).

Methods

Patients with HCM. The study cohort included 61 unrelated patients (age 38 ± 14 years; 72% male) undergoing PET for evaluation of symptoms such as dyspnea (n = 24), angina (n = 12), or risk stratification (n = 25). The diagnosis of HCM was based on 2-dimensional echocardiographic evidence of a hypertrophied LV (maximal wall thickness ≥15 mm) in the absence of any other cardiac or systemic cause of LV hypertrophy (1). Patients were enrolled from a large (n > 1,000) HCM population followed at our institution, among the 325 patients who completed an 8-gene mutational panel by December 2009. Children age <15 years and potentially child-bearing female patients were excluded. Although representing approximately 25% of potentially eligible patients, the study group is considered representative of the whole HCM cohort, based on age, sex, New York Heart Association (NYHA) functional class, prevalence of sarcomere gene mutations, and clinical status (10,12) (Table 1).

For all patients, informed consent was obtained, and the study protocol was approved by the local ethics committee. In all patients, epicardial coronary artery disease was excluded at the time of enrollment by maximal, symptom-limited cycloergometer echocardiography (performed routinely in our patients with HCM), followed by coronary angiography in patients with a positive or dubious test result. Patients with angina on effort were also studied invasively. Overall, coronary angiography was performed in 16 of the 61 study patients (26%) and was negative in each case. No evidence of regional perfusion defects on PET were observed, and the microvascular abnormalities were diffused to the whole LV (2–4).

Echocardiography. Comprehensive 2-dimensional and Doppler echocardiographic studies were performed in each patient using commercially available instruments. LV hypertrophy was assessed by 2-dimensional echocardiography, and the site and extent of maximal wall thickness were identified (12). Peak instantaneous LV outflow gradient, due to mitral valve systolic anterior motion and mitral-septal contact, was estimated with continuous wave Doppler under basal conditions (12).

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), and thin filament proteins (troponin T, troponin I, alpha-tropomyosin, and alpha-actin). Direct DNA sequencing was accomplished with the ABI-Prism 3730 (Applied Biosystems, Foster City, California), as previously described (10). Every variant identified was confirmed by direct sequencing and, whenever possible, by restriction enzyme digestion (10). Novel mutations were considered as potentially disease causing only if they were absent in at least 300 unrelated chromosomes from adult, ethnicity-matched healthy controls and produced an amino acid change in a highly conserved residue among species and isoforms (13). The Alamut software version 1.5e (Interactive Biosoftware, Rouen, France) was used for interpretation and reporting of unclassified variants.

All patients with HCM with a negative sarcomere gene test in the present study were screened for Fabry disease. In men, leukocyte alfa-galactosidase (alfa-GLA) activity was assessed, followed by genetic testing for alfa-GLA mutations by automatic gene sequencing when positive; women were assessed directly by genetic screening for alfa-GLA gene mutations (1). None of the patients included in this study proved affected.

Positron emission tomography. All PET scans were performed in the nuclear medicine laboratory of the Department of Clinical Physiopathology, Careggi University Hospital in Florence, Italy, between September 2003 and December 2008, after an appropriate period of pharmacological wash-out for patients receiving pharmacological treatment, with the exception of amiodarone (14,15). Patients were positioned on the couch of the PET scanner (General Electric Advance PET, Milwaukee, Wisconsin), and a 5-min transmission scan was recorded for subsequent attenuation correction of emission data. Then near-maximal hyperemia was induced by intravenous administration of dipyridamole (0.56 mg per kg of body weight over 4 min) (15). Three minutes following the end of dipyridamole infusion, a bolus of 370 MBq of ^{13}N-ammonia diluted in 10 ml of saline was injected intravenously over a period of 15 to 20 s and followed by a 10-ml saline flush at a rate of 2 ml/s. A dynamic scan with 15 frames of increasing duration was acquired for 4 min, followed by a prolonged static acquisition of 15 min. Data were analyzed with an operator

Table 1	General Features of the 61 Study Patients With HCM			
	Overall (N = 61)	Genotype Positive (n = 42)	Genotype Negative (n = 19)	p Value
Male	44 (72)	29 (69)	15 (79)	0.42
Age at enrollment, yrs	38 ± 14	39 ± 14	35 ± 15	0.34
Age at diagnosis, yrs	35 ± 12	34 ± 13	31 ± 14	0.41
Family history of HCM	25 (41)	17 (40)	8 (42)	0.90
Family history of juvenile sudden death	9 (15)	5 (12)	4 (21)	0.44
NYHA functional class at enrollment	1.5 ± 0.6	1.5 ± 0.6	1.4 ± 0.4	0.32
I	37 (61)	26 (62)	11 (58)	
II	21 (34)	13 (31)	8 (42)	0.58
III	3 (5)	3 (7)	0	
Syncope	16 (26)	11 (26)	5 (26)	0.99
Atrial fibrillation	13 (21)	11 (26)	2 (10)	0.44
Paroxysmal	8 (13)	7 (17)	1 (5)	0.38
Persistent/permanent	5 (8)	4 (9)	1 (5)	0.42
Implantable cardioverter-defibrillator	16 (26)	13 (31)	3 (16)	0.34
Medical treatment	49 (80)	35 (83)	14 (73)	0.31
Beta-blockers	41 (67)	29 (69)	12 (63)	0.58
Verapamil	6 (10)	5 (12)	1 (5)	0.27
Amiodarone	7 (12)	5 (12)	2 (10)	0.78
ACE inhibitors	5 (8)	4 (10)	1 (5)	0.36
Left atrium, mm	40 ± 7	40 ± 7	39 ± 9	0.49
Maximal LV wall thickness, mm	25 ± 6	25 ± 5	26 ± 8	0.55
Site of maximum LV wall thickness				
Septum	58 (95)	40 (95)	18 (95)	0.9
Other	3 (5)	2 (5)	1 (5)	
LV end-diastolic diameter, mm	43 ± 5	44 ± 5	41 ± 6	0.13
LV end-systolic diameter, mm	24 ± 6	25 ± 6	23 ± 5	0.14
LV ejection fraction, %	71 ± 9	70 ± 9	72 ± 8	0.89
With LVOT obstruction at rest (≥30 mm Hg)				0.67
Mitral regurgitation (1+ to 4+)	1.4 ± 0.8	1.4 ± 0.9	1.3 ± 0.6	0.66
Diastolic pattern				
Normal	26 (43)	16 (38)	10 (53)	
Delayed relaxation	18 (29)	15 (36)	3 (16)	0.25
Pseudonormal	8 (13)	4 (9)	4 (21)	
Restrictive	9 (15)	7 (17)	2 (10)	
Presence of cardiac MRI-LGE*	30/35 (86)	22/23 (96)	8/12 (67)	0.038
Cardiac MRI-LGE mass, % of whole LV*	6 ± 9	16 ± 15	17 ± 10	0.83

Values are n (%) or mean ± SD unless otherwise indicated. *Of 35 patients on whom cardiac magnetic resonance imaging (MRI) was performed (23 in the genotype-positive and 12 in the genotype-negative groups).

ACE = angiotensin-converting enzyme; HCM = hypertrophic cardiomyopathy; LGE = late gadolinium enhancement; LV = left ventricular; LVOT = left ventricular outflow tract; NYHA = New York Heart Association.

interactive computer program (PMOD cardiac modeling) (16). Briefly, anatomic images were reconstructed using the static acquisition and reoriented according to the heart axis. After reconstruction, the dynamic images were reoriented as well. On the short-axis slices, regions of interest were manually drawn, including: 1) the right ventricular cavity; 2) the LV cavity; and 3) the LV wall from the apex through the base (identified by the appearance of the membranous septum). The regions of interest were edited by the standard PMOD volume of interest (VOI) tool to derive the related VOIs. The 3 VOIs were then copied on all ^{13}N-ammonia dynamic images to extract the corresponding time-activity curves. The arterial input function was obtained from the right and left ventricular cavity time-activity curves. The myocardial uptake was derived from the LV wall VOI. Myocardial perfusion was calculated

from the dynamic data by fitting the arterial input function and tissue time-activity curves to a 2-tissue–compartment model for ^{13}N-ammonia developed by Hutchins et al. (17). Myocardial blood flow (MBF) following dipyridamole infusion for the entire LV (Dip-MBF) was obtained by volume-weighted averaging to the segment territories apex, septum, anterior, lateral, and inferior. All images studies were analyzed by 1 expert observer (R.S.) and blinded to patients' genetic, clinical, and echocardiographic data.

Cardiac MRI. Cardiac MRI was performed in 35 of the 61 study patients; the remaining 26 were excluded because of contraindications to cardiac MRI, such as an implantable cardioverter-defibrillator (n = 16), metallic clips (n = 3), or claustrophobia (n = 2), or because they refused the test (n = 5). Scans were performed using a

Table 2   Individual Patient Features, Dip-MBF, and Mutational Screening Results

Patient #	Age, yrs	Sex	Maximal LV Wall Thickness, mm	Dip-MBF, ml/min/g	Gene	cNomen	pNomen	Previously Described, Company or Author (Year) (Ref. #)
Genotype positive								
1	15	F	23	1.6	MYH7	c.1358G>A	p.Arg453His	Yu et al. (2005) (19)
2	17	M	32	1.3	MYH7	c.2573G>C	p.Arg858Pro	No
3	20	F	24	2.4	MYBPC3	c.2157_2158del	p.Cys719X	No
4	21	F	28	1.5	MYL2	c.173G>A	p.Arg58Gln	Flavigny et al. (1998) (20)
5	22	M	20	1.3	MYBPC3	c.772G>A	p.Glu258Lys	Niimura et al. (1998) (21)
6	23	M	27	1.7	MYH7	c.1816G>A	p.Val606Met	Watkins et al. (1992) (22)
7	23	M	39	1.3	MYBPC3	c.1020C>G	p.Tyr340X	No
8	23	M	23	1.2	MYBPC3	c.2429G>T	p.Arg810Leu	No
9	25	F	29	2.6	TNNT2	c.275G>A	p.Arg92Gln	Thierfelder et al. (1994) (23)
10*	25	F	36	1.9	MYBPC3	c.3192dup	p.Lys1065GlnfsX12	CardioGenomics
					MYBPC3	c.1112C>G	p.Pro371Arg	No
					MYH7	c.2606G>A	p.Arg869His	Van Driest et al. (2004) (24)
11	26	M	33	3.2	MYL2	c.173G>A	p.Arg58Gln	Flavigny et al. (1998) (20)
12*	26	F	27	1.3	TNNI3	c.257_258del	p.Ala86GlyfsX23	No
					MYBPC3	c.772G>A	p.Glu258Lys	Niimura et al. (1998) (21)
					MYH7	c.2606G>A	p.Arg869His	Van Driest et al. (2004) (24)
13	27	M	15	3.1	MYBPC3	c.1591G>C	p.Gly531Arg	CardioGenomics
14	30	M	22	1.7	MYH7	c.2893G>A	p.Glu965Lys	No
15*	33	M	17	1.1	MYBPC3	c.495G>C	p.Glu165Asp	No
					MYH7	c.2167C>T	p.Arg723Cys	Watkins et al. (1992) (22)
					MYH7	c.4363G>T	p.Glu1455X	No
16	35	M	27	1.5	MYBPC3	c.772G>A	p.Glu258Lys	Niimura et al. (1998) (21)
17	37	M	31	1.6	MYBPC3	c.2870C>G	p.Thr957Ser	No
18	37	M	30	1.3	MYBPC3	c.1624G>C	p.Glu542Gln	Carrier et al. (1997) (25)
19	37	M	26	2.8	MYBPC3	c.772G>A	p.Glu258Lys	Niimura et al. (1998) (21)
20	38	M	27	1.5	MYBPC3	c.3192dup	p.Lys1065GlnfsX12	CardioGenomics
21	39	M	23	1.3	MYH7	c.1063G>A	p.Ala355Thr	Richard et al. (2003) (31)
22	40	F	19	1.5	TNNT2	c.487_489del	p.Glu163del	Watkins et al. (1995) (22)
23*	41	M	25	1.4	MYBPC3	c.772G>A	p.Glu258Lys	Niimura et al. (1998) (21)
					MYBPC3	c.2728C>A	p.Pro910Thr	No
24	42	M	25	1.7	MYBPC3	c.772G>A	p.Glu258Lys	Niimura et al. (1998) (21)
25	42	M	26	1.1	MYBPC3	c.818G>A	p.Arg273His	No
26*	42	M	19	1.2	MYBPC3	c.772G>A	p.Glu258Lys	Niimura et al. (1998) (21)
					MYBPC3	c.1321G>A	p.Glu441Lys	No
27	43	F	22	1.5	MYH7	c.2594A>G	p.Lys865Arg	No
28	43	M	19	2.2	MYBPC3	c.772G>A	p.Glu258Lys	Niimura et al. (1998) (21)
29	43	M	22	0.6	MYBPC3	c.772G>A	p.Glu258Lys	Niimura et al. (1998) (21)
30	45	M	27	1.7	MYH7	c.1987C>T	p.Arg663Cys	Van Driest et al. (2004) (24)
31	47	M	18	2.1	MYBPC3	c.2258dup	p.Lys754GlufsX79	CardioGenomics
32	47	F	20	2.2	MYBPC3	c.636C>G	p.Ser212Arg	No
33	49	M	30	1.9	MYH7	c.1988G>A	p.Arg663His	Gruver et al. (1999) (26)
34	51	M	20	2.1	MYBPC3	c.1828G>C	p.Asp610His	No
35*	53	F	21	3.8	MYBPC3	c.2077G>T	p.Ala693Ser	No
					MYBPC3	c.2356G>T	p.Asp786Tyr	No
					MYBPC3	c.772G>A	p.Glu258Lys	Niimura et al. (1998) (21)
36	54	M	18	0.8	MYH7	c.2606G>A	p.Arg869His	Van Driest et al. (2004) (24)
37	56	F	21	1.5	TPM1	c.842T>C	p.Met281Thr	No
38	59	M	23	1.5	MYH7	c.2606G>A	p.Arg869His	Van Driest et al. (2004) (24)
39	62	M	16	1.5	MYH7	c.2606G>A	p.Arg869His	Van Driest et al. (2004) (24)
40	63	F	20	2.0	MYBPC3	c.1020C>G	p.Tyr340X	No
41	63	M	30	0.7	MYBPC3	c.772G>A	p.Glu258Lys	Niimura et al. (1998) (21)
42	63	M	30	1.2	MYBPC3	c.2905C>T	p.Gln969X	Yu et al. (1998) (27)

Table 2	Continued								
Patient #	Age, yrs	Sex	Maximal LV Wall Thickness, mm	Dip-MBF, ml/min/g	Gene	cNomen	pNomen		Previously Described, Author (Year) (Ref. #)
Genotype negative									
1	15	M	27	4.0	None	N/A	N/A		N/A
2	16	M	38	3.9	None	N/A	N/A		N/A
3	17	F	25	5.6	None	N/A	N/A		N/A
4	18	M	20	1.4	None	N/A	N/A		N/A
5	21	F	31	1.7	None	N/A	N/A		N/A
6	22	M	43	2.7	None	N/A	N/A		N/A
7	26	M	20	3.3	None	N/A	N/A		N/A
8	31	M	20	1.9	None	N/A	N/A		N/A
9	32	M	32	2.1	None	N/A	N/A		N/A
10	35	M	24	1.4	None	N/A	N/A		N/A
11	35	F	27	2.5	None	N/A	N/A		N/A
12	37	M	18	1.5	None	N/A	N/A		N/A
13	38	M	18	1.1	None	N/A	N/A		N/A
14	50	F	23	2.1	None	N/A	N/A		N/A
15	51	M	21	1.6	None	N/A	N/A		N/A
16	51	M	28	1.3	None	N/A	N/A		N/A
17	52	M	30	2.3	None	N/A	N/A		N/A
18	55	M	16	1.5	None	N/A	N/A		N/A
19	64	F	18	3.9	None	N/A	N/A		N/A

*Patients with complex genotypes.

Dip-MBF = dipyridamole myocardial blood flow; *MYBPC3* = myosin-binding protein C; *MYH7* = beta-myosin heavy chain; *MYL2* = myosin essential light chain; N/A = not applicable; *TNNI3* = troponin I; *TNNT2* = troponin T; *TPMI* = alpha-tropomyosin; other abbreviations as in Table 1.

Philips ACS-NT 1.5-T Gyroscan-Intera (Best, the Netherlands) using steady-state, free-precession breath-hold cines in sequential 10-mm short-axis slices (no gap) from the atrioventricular ring to the LV apex. LV end-diastolic and end-systolic volumes, LV mass, and LV wall thickness were calculated with commercially available workstations (Easy Vision 5.0 and View Forum, Philips Medical Systems). LGE images were acquired 10 to 15 min after intravenous administration of 0.2 mmol/kg gadolinium–diethylene triamine pentaacetic acid (Magnevist, Schering, Berlin, Germany) with breath-held segmented inversion-recovery sequence, acquired in the same orientations as the cine images (18). An inversion time scout was used initially to find the optimal inversion time to null normal myocardium (typically 240 to 300 ms). To ascertain the presence of LGE, all tomographic short-axis LV slices from base to apex were inspected visually to identify an area of completely nulled myocardium (18). Mean signal intensity (and SD) of normal myocardium was calculated, and a threshold of ≥2 SD exceeding the mean was used to define areas of LGE.
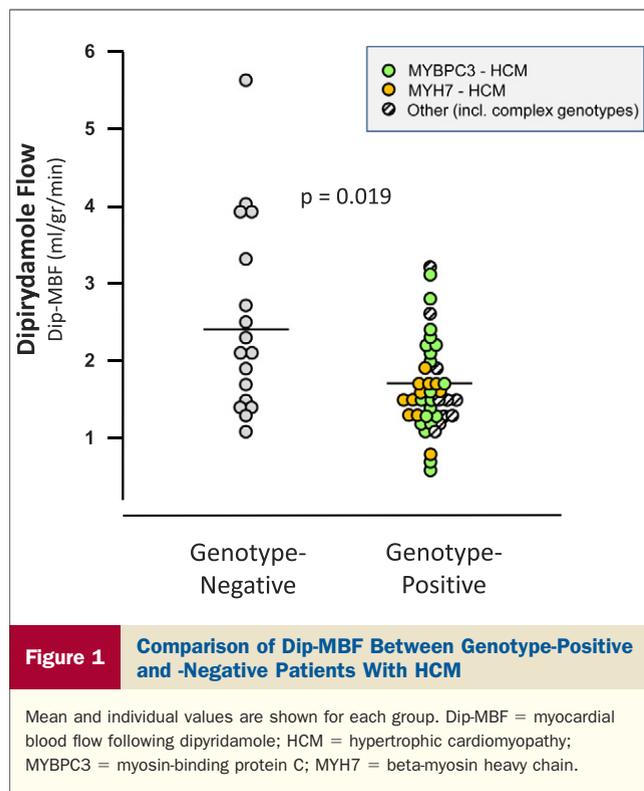
Statistical analysis. Unpaired Student *t* test or 1-way analysis of variance (ANOVA) was used for the comparison of normally distributed data. Chi-square or Fisher exact test were used to compare noncontinuous variables expressed as proportions. Relationships between independent variables were assessed with Spearman bivariate correlation analysis. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated using univariate logistic regression analysis.

All calculations were performed using SPSS software version 12.0 (SPSS Inc., Chicago, Illinois).

Results

General patient features. Of the 61 study patients, most (95%) were in NYHA functional class I or II (Table 1). Average LV wall thickness was 25 ± 6 mm (range 15 to 45 mm); among the 35 patients with cardiac MRI, LV mass index ranged from 66 to 369 g/m^2 (average 128 ± 68 g/m^2). Eight patients (13%) had dynamic LV outflow obstruction under resting conditions, 13 (21%) had a history of paroxysmal or permanent atrial fibrillation, and 16 patients (26%) had received an implantable cardioverter-defibrillator for primary (n = 13) or secondary (n = 3) prophylaxis of sudden cardiac death. None of the study patients had evidence of overt systolic dysfunction (i.e., LV ejection fraction ≤50%) at the time of the PET scan, although 4 patients had an LV ejection fraction in the low to normal range for HCM (55% to 65%).

Mutational analysis. Patients underwent comprehensive mutational analysis for 8 HCM-associated sarcomere genes. Overall, 53 mutations were identified in 42 of the 61 study patients (69%). Of these 42 genotype-positive patients, 36 had single, 2 had double, and 4 had triple mutations (Table 2) (19–27). Specifically, 20 patients had mutations in *MYBPC3* (48%), 11 in *MYH7* (26%), and 11 (26%) had mutations in the remaining 6 genes or had complex genotypes. Of the 53 mutations, 33 (62%) had been previously described and 20 (38%) were novel; among the latter, most (n = 13 [65%])

Figure 1   **Comparison of Dip-MBF Between Genotype-Positive and -Negative Patients With HCM**

Mean and individual values are shown for each group. Dip-MBF = myocardial blood flow following dipyridamole; HCM = hypertrophic cardiomyopathy; MYBPC3 = myosin-binding protein C; MYH7 = beta-myosin heavy chain.

had evidence of cosegregation within the family. In each case, the mutation affected a highly conserved residue, and the genetic defect was considered pathogenic with high likelihood by the Alamut software. Genotype-positive and genotype-negative patients were similar with regard to age at enrollment, sex, maximum LV thickness, left atrial size, prevalence of symptoms, ejection fraction, and mitral regurgitation under resting conditions (Table 1). Age at first diagnosis of HCM was also similar (34 ± 13 years vs. 31 ± 14 years, respectively; p = 0.41). Finally, the 2 subsets had comparable prevalence of family history for HCM, based on direct questioning and review of available clinical documentation (Table 1). Direct evaluation of first-degree relatives was not performed.

PET study results. Blood pressure measured at the time of PET scan was comparable in the genotype-positive and genotype-negative patient subsets, both at baseline (systolic 122 ± 15 mm Hg vs. 116 ± 13 mm Hg, p = 0.14; diastolic 74 ± 8 mm Hg vs. 72 ± 9 mm Hg, p = 0.39, respectively) and following dipyridamole infusion (systolic 120 ± 14 mm Hg vs. 113 ± 15 mm Hg, p = 0.09; diastolic 71 ± 8 mm Hg vs. 70 ± 10 mm Hg, p = 0.67, respectively). Mean Dip-MBF for the whole LV in the 61 study patients was 1.9 ± 0.9 ml/min/g, ranging from 0.6 to 5.6 ml/min/g. Twenty-one patients were in the lowest tertile of Dip-MBF (<1.5 ml/min/g), indicative of severe CMD, and 20 patients each were in the intermediate and highest tertiles (1.5 to 2.0 ml/min/g and >2.0 ml/min/g, respectively). Dip-MBF showed a weak inverse relation to age at enrollment (correlation coefficient −0.28, p = 0.027)

but had no relationship with sex, NYHA functional class, maximum LV thickness, or presence of LV outflow obstruction (p > 0.8 for all). There was no significant difference in flow based on septal morphology (Dip-MBF 1.7 ± 0.7 ml/min/g, 1.9 ± 0.8 ml/min/g, and 2.4 ± 1.7 ml/min/g in patients with reverse curvature, sigmoid, and apical subtypes, respectively, p = 0.11). Furthermore, we observed no difference in Dip-MBF between patients in chronic treatment with beta-blockers or verapamil (1.9 ± 0.8 ml/min/g vs. 2.1 ± 1.1 ml/min/g, respectively, p = 0.32).

Relevance of mutational status to Dip-MBF. Despite similar baseline features, Dip-MBF in genotype-positive patients averaged only 1.7 ml/min/g (range 0.6 to 3.8 ml/min/g) and was significantly lower than that in genotype-negative patients (2.4 ml/min/g; range 1.1 to 5.6 ml/min/g; p = 0.019) (Fig. 1). Only 24% of genotype-positive patients with HCM were in the highest tertile of Dip-MBF (>2.0 ml/min/g), compared with 53% of patients with a negative genetic test; conversely, 40% of genotype-positive patients were in the lowest tertile (Dip-MBF <1.5 ml/min/g), compared with 21% of genotype negative (overall p = 0.07). Of note, among 5 patients in the pediatric age range (<18 years), the 2 with sarcomere myofilament mutations (i.e., *MYH7*-Arg453His and *MYH7*-Arg858Pro) had blunted Dip-MBF values of 1.3 and 1.6 ml/min/g, whereas the 3 genotype-negative patients had normal values ranging from 3.9 to 5.6 ml/min/g (Fig. 2).

Overall, a Dip-MBF <1.5 ml/min/g had an 81% positive predictive value with regard to genotype-positive status. Conversely, a Dip-MBF in the highest tertile (>2.0 ml/min/g) was associated with a 72% reduction in likelihood of genotype-positive status, compared with values <2.0 ml/min/g (HR: 0.28, 95% CI: 0.09 to 0.88, p = 0.03). Stepwise logistic regression analysis showed Dip-MBF to be the only independent predictor of genotype-positive status (Table 3): values <1.5 ml/min/g implied a 3.5-fold increase in likelihood of carrying 1 or more myofilament gene mutations (HR: 3.52, 95% CI: 1.05 to 11.7, p = 0.04).

Among genotype-positive patients, there was no difference in average Dip-MBF with respect to the 2 major genes (1.7 ± 0.6 ml/min/g for *MYBPC3* vs. 1.5 ± 0.3 ml/min/g for *MHY7*, respectively; p = 0.23). However, patients with *MYBPC3* mutations showed a broad spectrum of flow values (range 0.6 to 3.1 ml/min/g), whereas those with *MYH7* mutations a narrow range that was constantly below 2 ml/min/g (range 0.8 to 1.9 ml/min/g) (Fig. 1).

Late gadolinium enhancement. Of the 35 patients with cardiac MRI, 30 (86%) showed evidence of LGE, averaging 31 ± 37 ml in volume or 6 ± 9% of the whole LV (Table 1). Prevalence of LGE was greater in the genotype-positive subgroup (22 of 23 [96%] compared with 8 of 12 [67%] in the genotype-negative group; p = 0.038). However, there was no significant difference in LGE volume between the 2 groups (Table 1).
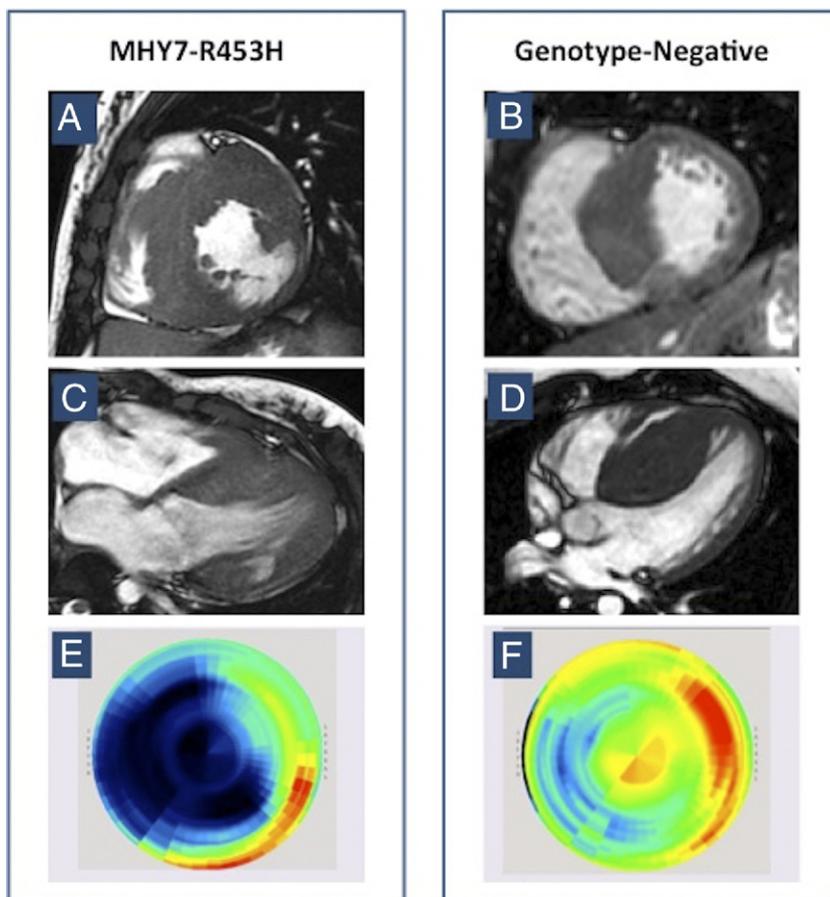
Figure 2   Genetic Status and Microvascular Function in 2 Adolescents With HCM

(A and B) Cardiac magnetic resonance short-axis and **(C and D)** 4-chamber views in a 15-year-old girl with HCM-causing *MHY7* mutation **(left)** and 16-year-old boy with negative genetic screening **(right)**. Both patients have the typical HCM phenotype with severe asymmetric hypertrophy localized at the ventricular septum. Polar maps obtained with positron emission tomography after dipyridamole infusion show severe microvascular dysfunction in the **(E)** genotype-positive (mean Dip-MBF 1.6 ml/min/g) but not in the **(F)** genotype-negative patient, in whom myocardial flow appears to be preserved (mean Dip-MBF 3.9 ml/min/g). Abbreviations as in Figure 1.

Discussion

Genetic status and microvascular dysfunction in HCM.
HCM is the most common genetic cardiac disease, characterized by heterogeneous morphologic expression and clinical course (1,3,9,11,12,18). Mutations in genes coding for myofilament contractile proteins of the cardiac sarcomere characterize the most common genetic subtype of HCM, with a 30% to 65% prevalence in various cohort studies (10,28–31). As novel genes associated with HCM are being discovered, these only account for a small fraction of individual cohorts (29,32). Therefore, a considerable proportion of patients prove genotype negative even after the most comprehensive mutational screening (19).

Recent evidence shows that patients in whom HCM is associated with proven sarcomere myofilament mutations have substantial prevalence of disease progression, LV dysfunction, and likelihood of end-stage development (10,33,34), which is not as common among genotype-negative patients (10). Such risk becomes particularly

marked in the presence of multiple mutations (35–37). On the other hand, there is substantial evidence suggesting a pivotal role of microvascular ischemia in determining LV remodeling and replacement fibrosis—2 hallmarks of end-stage HCM (2,11). Importantly, severe CMD is an important predictor of adverse outcome because of heart failure–related complications in these patients (3,4). Therefore, we hypothesized that the more frequent occurrence of disease progression and LV dysfunction observed in patients with HCM with sarcomere myofilament mutations might be related to more prevalent and severe impairment of the coronary microcirculation (9).

The findings of the present investigation support this hypothesis. In our 61 patients assessed by mutational analysis for the most frequent HCM-causing genes, Dip-MBF was more frequently and severely impaired in genotype-positive individuals, compared with the genotype negative, despite similar baseline features including age, symptoms, degree of hypertrophy, and functional LV char-

Table 3	Logistic Regression Analysis Assessing Predictors of Genotype-Positive Status	
	p Value	Hazard Ratio (95% Confidence Interval)
Dip-MBF (per ml/min/g increase)	0.007	0.34 (0.15–0.74)*
Left atrial diameter (per mm increase)	0.82	
Age	0.72	
Female sex	0.24	
Family history of HCM	0.77	
Family history of sudden cardiac death	0.52	
NYHA functional class	0.13	
Atrial fibrillation	0.77	
LV outflow obstruction	0.96	
Maximum LV thickness	0.72	
End-diastolic LV diameter (per mm increase)	0.58	

*Preserved Dip-MBF is inversely associated with the likelihood of carrying a sarcomere myofilament gene mutation.
Abbreviations as in Tables 1 and 2.

acteristics such as ejection fraction, mitral regurgitation, and prevalence of outflow obstruction. Dip-MBF averaged only 1.7 ml/min/g in genotype-positive patients with HCM, compared with 2.4 ml/min/g in genotype negative. Genotype-positive patients were one-half as likely to be in the highest tertile of flow for the whole group (Dip-MBF >2.0 ml/min/g), compared with genotype negative, but twice as likely to be in the lowest tertile (Dip-MBF <1.5 ml/min/g), reflecting profound microvascular dysfunction. Specifically, all patients with *MHY7* mutations showed a blunted response to dipyridamole, with flow values consistently below the 2 ml/min/g threshold. In addition, we observed a higher prevalence of LGE among genotype-positive patients in our cohort, suggesting that the milieu associated with myofilament mutations may be more susceptible to replacement fibrosis and LV remodeling (6,8,38).

Two potentially important concepts can be derived from our observations. The first is that severe impairment of microvascular function is not an obligate consequence of the HCM phenotype; in genotype-negative patients, a broad range of Dip-MBF values was observed, including patients with relatively preserved flow despite massive hypertrophy, as shown in Figure 2. The second is that genotype-positive patients with HCM exhibited a more stereotypical response to dipyridamole, which is, with few exceptions, severely impaired. This supports the idea that the specific genetic defect causing HCM may represent a major determinant of microvascular remodeling, as originally hypothesized. In a broader prospective, our findings suggested that arteriolar remodeling is regulated by selective activation of molecular pathways that are largely independent of hypertrophy itself, consistent with the great heterogeneity of microvascular involvement observed in patients with secondary LV hypertrophy (7). For example, microvascular remodeling is an established feature of hypertensive heart disease and appears to be finely regulated at the molecular level, whereas aortic stenosis seems to cause significantly milder degrees of

microvascular remodeling, despite similar or greater magnitude of LV hypertrophy (7).

Of note, we observed an inverse relationship between age at enrollment and Dip-MBF. However, such a relationship was very weak (correlation coefficient −0.28, p = 0.027), suggesting that duration of disease is not a major determinant of microvascular dysfunction. Such a concept is consistent with previous work by our group (4,15), demonstrating that severe CMD leading to LV remodeling and end-stage disease may occur at any age in patients with HCM.

Clinical implications. From a clinical perspective, sarcomere myofilament mutations seem to confer a distinctly increased risk of disease progression and heart failure–related complications in patients with HCM, mediated by severe microvascular dysfunction, myocardial ischemia, fibrosis, and remodeling (2). Patients with complex genotypes, harboring double or triple genetic defects, by virtue of their severe phenotype and clinical course, represent extreme paradigms supporting an association between genetic status and outcome (35–37). Consequently, our findings emphasize the clinical relevance of systematic genetic screening in HCM populations, potentially guiding surveillance strategies and early medical intervention with antiremodeling therapies aimed at preventing disease progression in predisposed individuals (2,19,29). Preliminary evidence suggests the possibility of targeting CMD by specific pharmacological interventions in patients with secondary LV hypertrophy (39,40), and this appears promising for patients with HCM as well. However, our findings suggest that selection of individuals with a homogeneous and well-defined genetic background, particularly aimed at individuals with sarcomere myofilament mutations, is mandatory to obtain meaningful and reproducible results (10).

Potential mechanisms. The link between sarcomere gene mutations and CMD remains elusive (9). Patients with HCM exhibit striking anatomic remodeling of the intramural coronary arterioles, predominantly characterized by smooth muscle hypertrophy in the tunica media leading to lumen reduction and inability to properly vasodilate in response to physiological or pharmacological stimuli (7). However, none of the cells involved in microvascular remodeling are known to express the sarcomere proteins that are mutated in HCM (9). Therefore, it is difficult to postulate a direct effect of HCM-causing mutations on small vessels. One possibility is that microvascular remodeling might represent secondary, maladaptive changes triggered by abnormal LV wall stress or other hemodynamic abnormalities, similar to those found in arterial hypertension (7). Such a hypothesis, however, fails to explain CMD observed in patients with very mild hypertrophy, including young children, with normal LV function and no evidence of outflow obstruction (2,9).

An alternative, although not mutually exclusive, explanation is represented by the possibility of a developmental origin of microvascular abnormalities in patients with HCM

(9). The coronary circulation develops from proepicardial cells that colonize the embryonic heart when the cardiomyocytes have already begun to contract (41). The exposure of coronary precursors to abnormal mechanical stimuli, resulting from myofilament mutation effect on sarcomere function, may be capable of influencing their development (9,41,42). Of note, the same mechanism may explain other phenotypic expressions of HCM involving systems of proepicardial origin, such as mitral valve abnormalities and irregular development of the fibrous heart skeleton, producing disarray (9,42). Further studies are needed to test such hypotheses. Intriguingly, however, the role of developmental influences on the phenotype of cardiomyopathy patients is beginning to surface for other conditions such as LV noncompaction and arrhythmogenic right ventricular cardiomyopathy (41,43).

Study limitations. We are aware that to consider patients with none of the known HCM-causing mutations as a single group represents an unavoidable oversimplification of the present study, calling for further research in this area. Genotype-negative HCM represents a composite entity, probably comprising a multitude of rare, heterogeneous, and yet-to-be identified HCM-susceptibility genes. To date, more than 20 different candidate genes have been identified, including titin, muscle LIM protein, telethonin, myozenin, vinculin, and actinin (19). Nevertheless, the prevalence of mutations in these newly discovered genes is very low (<5% combined); therefore, even a very extensive screening would not have altered our study results significantly. It is hoped that the yield of genetic testing in genotype-negative HCM will radically improve in the near future with the advent of next-generation sequencing techniques, allowing a whole-genome approach (28).

Automatic DNA sequencing, used in the present study for mutational analysis and representing the standard in HCM genetic testing, is not capable of detecting large insertions/deletions. Therefore, we cannot exclude that a subset of genotype-negative patients may carry such defects within sarcomere myofilament genes that were not recognized. Nevertheless, in the only study specifically addressing this issue, large deletions and duplications in *MYBPC3* and troponin T appeared to be exceedingly rare (44).

Conclusions

Sarcomere myofilament mutations are associated with severe blunting of microvascular function in patients with HCM, to a degree that is not observed in genotype-negative individuals. These findings suggest that sarcomere gene mutations might be directly involved in the generation of adverse remodeling of the microcirculation, ischemia, and replacement fibrosis in HCM, accounting for the greater prevalence of ventricular dysfunction and cardiac failure compared with genotype-negative disease.

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