Significance of Sarcomere Gene Mutations Analysis in the End-Stage Phase of Hypertrophic Cardiomyopathy

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End-stage hypertrophic cardiomyopathy (ES-HC) has an ominous prognosis. Whether genotype can influence ES-HC occurrence is unresolved. We assessed the spectrum and clinical correlates of HC-associated mutations in a large multicenter cohort with end-stage ES-HC. Sequencing analysis of 8 sarcomere genes (MYH7, MYBPC3, TNNI3, TNNT2, TPM1, MYL2, MYL3, and ACTC1) and 2 metabolic genes (PRKAG2 and LAMP2) was performed in 156 ES-HC patients with left ventricular (LV) ejection fraction (EF) <50%. A comparison among mutated and negative ES-HC patients and a reference cohort of 181 HC patients with preserved LVEF was performed. Overall, 131 mutations (36 novel) were identified in 104 ES-HC patients (67%) predominantly affecting MYH7 and MYBPC3 (80%). Complex genotypes with double or triple mutations were present in 13% compared with 5% of the reference cohort ($p = 0.013$). The distribution of mutations was otherwise indistinguishable in the 2 groups. Among ES-HC patients, those presenting at first evaluation before the age of 20 had a 30% prevalence of complex genotypes compared with 19% and 21% in the subgroups aged 20 to 59 and ≥60 years ($p = 0.003$). MYBPC3 mutation carriers with ES-HC were older than patients with MYH7, other single mutations, or multiple mutations (median 41 vs 16, 26, and 28 years, $p \leq 0.001$). Outcome of ES-HC patients was severe irrespective of genotype. In conclusion, the ES phase of HC is associated with a variable genetic substrate, not distinguishable from that of patients with HC and preserved EF, except for a higher frequency of complex genotypes with double or triple mutations of sarcomere genes. **(Am J Cardiol 2014;114:769−776)**

The end-stage (ES) phase of hypertrophic cardiomyopathy (HC) carries an ominous prognosis because of high rates of refractory heart failure and sudden arrhythmic death and represents the sole indication for heart transplantation in HC.[1−6] Several different mechanisms have been proposed to explain the evolution toward ES, such as progressive cardiomyocyte energy depletion, microvascular ischemia, and replacement fibrosis although it remains largely unresolved.[7−11] It has also been hypothesized that development of ES may be heavily influenced by the specific genetic background. Indeed, multiple sarcomere gene mutations are associated with early onset of HC disease and particularly severe phenotypes, including evolution toward the ES.[12−17] To date, however, systematic studies on the genetic background of ES-HC are limited, probably because of the relative rarity of this condition in most HC cohorts.[18,19] Therefore, to adequately investigate this issue, we assessed the prevalence and spectrum of sarcomere gene mutations in the largest multicenter cohort of patients with ES-HC reported to date, with respect to phenotypic expression, clinical course, and outcome. Moreover, a comparison of the genetic substrate in ES-HC with a subset of HC patients with preserved left ventricular (LV) ejection fraction (EF) was assessed.

Methods

In this multicenter cross-sectional and longitudinal study, we retrospectively identified 156 patients diagnosed with

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Table 1
Clinical and echocardiographic characteristics of the overall ES-HC population and of patients with negative genotype, single mutations and complex genotype

Variable	Overall	Negative Genotype	Single Mutations	Multiple Mutations	p Value
	(n = 156)	(n = 52)	(n = 83)	(n = 21)	
Proband	130 (83%)	50 (96%)	63 (76%)	17 (81%)	0.008
Male sex	86 (55%)	29 (56%)	43 (52%)	14 (67%)	0.470
Age at HC diagnosis (years)	30 (16–45)	34 (17–50)	30 (16–47)	28 (17–33)	0.271
Age at 1st evaluation (years)	44 (30–55)	45 (32–55)	45 (31–55)	40 (26–46)	0.317
Age at ES diagnosis (years)	45 (35–57)	47 (33–58)	47 (36–58)	42 (35–51)	0.342
Family history of HC	97 (63%)	26 (50%)	59 (71%)	12 (60%)	0.047
Family history of SD	57 (37%)	16 (31%)	35 (42%)	6 (30%)	0.326
Family history of ES HC	45 (29%)	11 (21%)	27 (33%)	7 (35%)	0.300
Echocardiographic data at ES diagnosis					
Maximal wall thickness (mm)	17 (14–22)	17 (14–22)	17 (14–20)	17 (15–19)	0.702
LV ED cavity dimension (mm)	55 (49–59)	55 (49–59)	50 (47–55)	55 (51–61)	0.019
LVEF (%)	45 (34–48)	45 (34–48)	45 (36–49)	42 (33–45)	0.425

Note: Data are expressed as median (IQR).
ED = end-diastolic; EF = ejection fraction; ES = end-stage; HC = hypertrophic cardiomyopathy; LV = left ventricular; SD = sudden death.

ES-HC (130 index cases and 26 family members) from January 1981 to June 2010 and genetically screened between January 2007 and June 2010 at 6 referral centers in Italy and 2 in the United States. Five of these centers (Dipartimento di Medicina Specialistica, Diagnostica e Sperimentale, Alma Mater Studiorum, Università di Bologna, Italy; Dipartimento di Cardiologia, Seconda Università degli Studi, Napoli, Italy; Dipartimento Cardiovascolare, Ospedali Riuniti, Bergamo, Italy; Hypertrophic Cardiomyopathy Center, Minneapolis Heart Institute Foundation, Minneapolis, MN; Tufts Medical Center, Hypertrophic Cardiomyopathy Center, Boston, MA) have dedicated heart failure management units including heart transplantation programs.

Diagnosis of HC was based on echocardiographic and/or cardiovascular magnetic resonance imaging documentation of a hypertrophied, nondilated LV, in the absence of other cardiac or systemic diseases that could produce the magnitude of LV hypertrophy evident.[20] ES-HC was defined as 2D echocardiographic LV EF <50% at rest, reflecting global systolic dysfunction, at study entry or during follow-up.[3,4] Patients with previous surgical myectomy or alcohol septal ablation (n = 16), known atherosclerotic coronary artery disease (n = 9), or severe valvular heart disease (n = 8) were excluded.

A subset of 181 patients with HC and normal LV systolic function consecutively genotyped for 10 genes between January 2007 and June 2010 at Centro di Riferimento per le Cardiomiopatie, Azienda Ospedaliero-Universitaria Careggi, Firenze, Italy, was examined as a reference cohort.[14] Median age at first evaluation was 52 years (interquartile range [IQR] 36 to 63); 64 (35%) were female, median maximum LV wall thickness was 22 mm (IQR 19 to 27), left atrium diameter 45 mm (IQR 40 to 51); 34 (19%) had LV outflow tract obstruction in conditions at rest; 164 of 181 patients (91%) were in New York Heart Association (NYHA) class I to II; 25 (19%) were implanted with an implantable cardioverter-defibrillator (ICD) for primary or secondary protection from sudden death.

We considered echocardiographic studies performed at first evaluation at the referral center and first documentation of ES. Maximum wall thickness, LV end-diastolic cavity dimension, left atrial dimension, LV outflow tract obstruction, and LVEF were evaluated. LV hypertrophy was assessed with 2-dimensional echocardiography, and the site of maximum wall thickness was identified.

All patients underwent genetic testing for the 8 most frequently mutated sarcomeric genes associated with HC: cardiac beta-myosin heavy chain (MYH7, NM_000257.2), cardiac myosin-binding protein C (MYBPC3, NM_000256.3), troponin I (TNNI3, NM_000363.4), troponin T (TNNT2, NM_001001430.1), alpha-tropomyosin (TPM1, NM_000366.5), regulatory myosin light chain (MYL2, NM_000432.3), essential myosin light chain (MYL3, NM_000258.2), and alpha-cardiac actin (ACTC1, NM_005159.4) as well for lysosome-associated membrane protein 2 (LAMP2, NM_002294.2) and AMP-activated protein kinase (PRKAG2, NM_016203.3) as possible cause of HC phenocopies. Written informed consent was obtained in each case. Conventional DNA sequencing was performed using standard method.[14]

Each identified variant was confirmed by direct sequencing from an independent amplification product and, whenever possible, by restriction enzyme digestion. The significance of each variation was defined by the following criteria, although not always all of them present for each mutation: (1) absence in 300 adult control chromosomes from ethnically matched subjects, tested by sequencing; (2) minor allele frequency (MAF) <1%, collected from ESP (Exome Sequencing Project, *http://evs.gs.washington.edu/EVS/*, data release ESP6500SI-V2) and dbSNP137 (*http://www.ncbi.nlm.nih.gov/SNP/*), that includes 1000Genomes Project data; (3) evolutionary conservation of the nucleotide or amino acid, calculated by multiple alignments of 46 vertebrate species and measured by phyloP score; and (4) in silico functional prediction of effect by in silico tools: PolyPhen (*http://genetics.bwh.harvard.edu/pph/*), SIFT (*http://sift.jcvi.org/*), and MutationTaster (*http://neurocore.charite.de/MutationTaster*) for coding variants; a specific software Alamut 2.0 was used to search for splicing prediction. Moreover, the presence in the literature of identified