Supplementary material

Factors Influencing the Phenotypic Expression of Hypertrophic Cardiomyopathy in Genetic Carriers

METHODS OF THE SUPPLEMENTARY MATERIAL

METHODS

Cardiac Evaluation

Patients were interviewed and asked about symptoms, their history of hypertension, and regular physical exercise. Echocardiographic variables included the pattern of left ventricular hypertrophy; indexed left ventricular wall thickness; left ventricular mass (Devereaux); left ventricular systolic and diastolic diameters; left atrium diameter; left ventricular systolic and diastolic function (systolic dysfunction was considered if left ventricular ejection fraction was < 50%); left ventricular outflow tract gradient and the presence of mitral regurgitation. Left ventricular outflow tract obstruction was defined as a left ventricular outflow tract gradient > 30 mmHg. All patients were offered a 24-hour electrocardiogram-Holter and exercise test to complete sudden death risk stratification. Nonsustained ventricular tachycardia was assessed. Arrhythmic events and complications during follow-up were also recorded. Estimation of the sudden death risk rate at 5 years was calculated using the formula provided by O'Mahony et al.[1]

In the cohort there were 3 resuscitated cardiac arrests and 6 appropriate implantable cardioverter-defibrillator discharges, which were computed as sudden death equivalent for survival estimates.

After identification of the index case, family study was performed actively and all first-degree relatives were invited to participate in the cardiac evaluation and had the same opportunities to be screened.

The age of each event or age at last follow-up were used for survival analysis.

Hypertension was defined following the European Society of Hypertension and European Society of Cardiology recommendation.[2] At least 3 readings were taken for systolic blood pressure > 140 mmHg or diastolic blood pressure > 90 mmHg. Patients with a prior diagnosis of hypertension who were under chronic antihypertensive medication were considered as hypertensive for the purpose of the study.

Medication at the time of the first evaluation was recorded. Among hypertensive patients, medication consisted of beta-blockers in 43 (68.3%), calcium antagonists in 20 (31.7%), an angiotensin-converting enzyme inhibitor or angiotensin receptor blocker in 15 (23.8%), alfa$_1$-blockers in 16 (25.4%), and diuretics in 27 (42.9%). More than 1 antihypertensive drug was used in 28 patients (44.4%).

All of the patients and their relatives were informed about their disease and gave written consent. This study was approved by the local ethics committee.

Genetic Tests

Genomic DNA was extracted from peripheral blood samples using standard protocols. Sequencing of the most prevalent sarcomeric genes (2-5) included in all *MBPC3* and *MYH7* was performed using BigDye v1.1 chemistry in an ABI3130 analyzer (Applied Biosystems, Foster City) in 58 (80.5%) probands. In 23 (31.9%) of these, a genetic study was completed with sequencing of *TNNT2, TNNI3*, and *TPM1*. In the remaining 14 probands, (19.5%), the genetic study was considered complete when 1 of the founder mutations was identified. A predictive test was then offered to all first-degree relatives who were then screened for the mutation identified in the proband.

REFERENCES

1. O'Mahony C, Jichi F, Pavlou M, et al. A novel clinical risk prediction model for sudden cardiac death in hypertrophic cardiomyopathy (HCM risk-SCD). *Eur Heart J.* 2014;35:2010-2020.

2. Mancia G, De Backer GD, Dominiczak A, et al. 2007 Guidelines for the management of arterial hypertension. The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Eur Heart J.* 2007;28:1462-1536.

Table 1 of the supplementary material

Frequency of Affected Carriers Vs Non-affected Carriers in the Different Group of Individuals

	Sedentary		Moderate		Intense		Not available		Total	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
No HT	67/19 (58.8%/16.7%)	35/36 (34.3%/35.3%)	10/3 (52.6%/15.8%)	2/4 (33.3%/66.7%)	8/2 (57.1%/14.3%)	0	2/1 (28.6%/14.3%)	1/7 (11.1%/77.8%)	87/25 (56.1%/16.1%)	38/47 (32.2%/39.8%)
HT	25/0 (21.9%/0%)	23/4 (22.5%/3.9%)	4/0 (21.1%/0%)	0	3/1 (21.5%/7.1%)	0	2/0 (28.6%/0%)	1/0 (11.1%/0%)	34/1 (21.9%/0.6%)	24/4 (20.3%/3.4%)
Not available	3/0 (2.6%/0%)	3/1 (2.9%/1.0%)	1/1 (5.3%/5.3%)	0	0	0	2/1 (28.6%/14.3%)	0	6/2 (3.9%/1.3%)	3/1 (2.5%/0.8%)
Total	95/19 (83.3%/16.7%)	61/41 (59.8%/40.2%)	15/4 (78.9%/21.1%)	2/4 (33.3%/66.7%)	11/3 (78.6%/21.4%)	0	6/1 85.7%/14.3%)	2/7 (22.2%/77.8%)	127/28 (81.9%/18.1%)	66/52 (55.9%/44.1%)

Each box represent “affected carrier/non-affected carrier”

HT, hypertension.

Table 2 of the supplementary material

Clinical Characteristics of Individuals Who Perform Intense or Competitive Exercise

	Age at evaluation	Gender	Age at diagnosis	Reason for diagnosis	Type of sport	Duration of exercise	Max LVH	Obstruction	LVEDd	LA	Syncope	NYHA III-IV	AF	Events
1	26	Male	16	Symptoms	Cycling and weight lifting (>14 hours/week)	Continues to practice sports to date	25	No	45	39	No	No	No	ICD implanted. Device complications related to weight lifting, (last FU 27 years old)
2	63	Male	51	Family screening	Competitive tennis (>10 hours/week)	Stopped tennis at 55 years old	22	Yes	44	34	No	No	No	No events (last FU 65 years old)
3	29	Male	27	Family screening	Running, swimming and cycling, training for military inclusion (>14 hours/week)	Reduced intensity at diagnosis, died 2 years later	22	No	65	55	No	Yes	No	Progressive dyspnea, dilatation, heart failure death at 29 years old
4	14	Male	14	Family screening	Soccer (>14 hours/week)	Continues to do sports to date	13	No	46	43	No	No	No	No events (last FU 15 years old)
5	17	Male	14	Incidental	Competitive Soccer and running (10 hours/week)	Reduced intensity	27	No	45	39	No	No	No	No events (last FU 17 years old)
6	39	Male	31	Symptoms	Soccer (>10 hours/week)	Reduced intensity	14	No	44	45	No	No	No	No events (last FU 41 years old)
7	60	Male	47	Symptoms	Highly physical work + running (> 14 hours/week)	Stopped sports at diagnosis (aged 47)	21	Yes	44	48	No	Yes	Yes	ICD implanted 49 (last FU 61 years old)

8	62	Male	44	Family screening	Tennis and cycling (> 10 hours/week)	Reduced intensity (aged 52 years)	22	No	42	48	Yes	No	Yes	ICD implanted 52 (last FU 63 years old)
9	56	Male	45	Symptoms	Professional boxing, running and cycling (>10 hours/week)	Reduced intensity before diagnosis (aged 49 years)	18	No	48	42	Yes	No	No	No events (last FU 59 years old)
10	41	Male	25	Family screening	Soccer and running (> 10 hours/week)	Stopped sports at diagnosis (aged 25 years old)	28	No	45	30	Yes	No	No	ICD recommended (last FU 42 years old)
11	66	Male	61	Symptoms	Cycling and running (> 10 hours/week)	Stopped sports before diagnosis (aged 35 years)	16	Yes	47	50	Yes	Yes	No	No events (last FU 67 years old)
12	24	Male	Non-affected	Non-affected	Professional athlete (> 14 hours/week)	Continues to do sports to date	10	No	44	35	No	No	No	No events (last FU 26 years old)
13	22	Male	Non-affected	Non-affected	Professional soccer (> 14 hours/week)	Continues to do sport to date	11	No	49	31	No	No	No	No events (last FU 22 years old)
14	66	Male	Non-affected	Non-affected	Professional soccer + running and cycling (> 14 hours/week)	Reduced intensity before diagnosis (aged 34 years)	10	No	42	36	No	No	No	No events (last FU 67 years old)

AF, atrial fibrillation; FU, follow-up; ICD, implantable cardioverter-defibrillator; LA, left atrium; LVEDd, left ventricular end diastolic diameter; LVH, left ventricular hypertrophy; NYHA, New York Heart Association class

Table 3 of the supplementary material.

Results of Multivariable Analysis of Survival (Cox and Laplace). Statistical Significant Variables Associated With the Event

		Cox (HR)		Laplace p50 (median) (years)	
Event	**variable**	**value (95%CI)**	**sig. (*P*)**	**value (95%CI)**	**sig. (*P*)**
Stroke					
	LA diameter	1.10 (1.01-1.20)	.042	-0.93 (-1.50 to -0.36)	< .001
	Type of gene			-20.71 (-33.23 to -8.20)	< .001
AF					
	LA diameter	1.09 (1.05-1.14)	< .001	-1.28 (-1.68 to -0.88)	< .001
NYHA III-IV					
	LA diameter	1.07 (1.02-1.12)	.002	-1.02 (-1.68 to -0.36)	.002
	Physical activity			-14.11 (-24.10-4.11)	.006
	Male gender			9.97 (1.21-18.70)	.026
	HT			10.38 (1.71-19.66)	.019
SD					
	LVH	1.10 (1.01-1.20)	.039		
	LA diameter	1.07 (1.03-1.10)	< .001		
Combined event					
	LA diameter	1.07 (1.03-1.10)	< .001	-0.70 (-1.19 to -0.20)	.006

95%CI, 95% confidence interval; AF, atrial fibrillation; HT, hypertension; LA, left atrial diameter (mm); LVH, left ventricular hypertrophy; NYHA, New York Heart Association class; SD, sudden death.
Combined event, includes stroke, AF, NYHA III-IV. SD equivalent includes sudden death, resuscitated cardiac arrest and implantable cardioverter-defibrillator therapy. LVH, indexed maximal left ventricular hypertrophy (mm/m^2). Type of gene where mutation is located (*MYBPC3* vs *MYH7*).
Variables included in the model: physical activity, gender, HT, Type of gene, obstruction, maximal wall thickness (indexed in mm/m^2), and LA diameter (mm).