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The Influence of Genotype on the Cardiopulmonary Test Response in Patients Affected by Hypertrophic Cardiomyopathy

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Abstract: In hypertrophic cardiomyopathy (HCM), the presence of pathogenic/likely pathogenic (P/LP) disease-causing genetic variants may indicate a worse prognosis. Few data exist on the effects of these genetic variants on cardiopulmonary exercise test (CPET) performance in HCM patients. We analysed asymptomatic and slightly symptomatic HCM patients (NYHA I-II) whose genetic analysis and CPET were available; at baseline, left ventricular function was normal and severe left ventricular outflow trait obstruction was excluded. Out of 120 HCM patients, we excluded 13 carrying variants of uncertain significance; of the remaining 107 patients, 54 were genotype negative [gene (−)], and 53 had a P/LP variant in sarcomeric genes [gene (+)]. Patients in the two groups had similar NYHA class, cardiovascular risk factors and echocardiographic characteristics. Gene (+) patients showed a lower peak VO_2% and O_2 pulse % ($p < 0.05$). Moreover, among gene (+), patients with P/LP variants in the so called "thin-filament" genes (*TNNT2, TPM1* and *MYL3*) had the poorest CPET results. In asymptomatic or slightly symptomatic HCM patients with similar echocardiographic characteristics, exercise tolerance is affected by the genetic background. Indeed, exercise capacity is poorer in gene (+) compared to gene (−) patients and those carrying P/LP variants in "thin-filament" genes show the worst performance.

Keywords: hypertrophic cardiomyopathy; genetic analysis; cardiopulmonary test; exercise tolerance

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1. Introduction

Hypertrophic cardiomyopathy is a primary cardiac disorder, defined as the presence of increased left ventricular wall thickness (≥15 mm) with or without right ventricular

hypertrophy, that is not solely explained by abnormal loading conditions [1,2]; lesser degrees of wall thickening (≥13 mm) are required for the diagnosis in family members [2]. HCM is the most common among inherited cardiac conditions, it is mainly transmitted in an autosomal dominant mode and genetic analysis is recommended in all patients fulfilling diagnostic criteria for HCM. The identification of a P/LP variant in the proband confirms the diagnosis of sarcomeric HCM and excludes the presence of phenocopies that could require a specific treatment. Family members should be screened whenever a P/LP variant is identified in the proband [2–8]. The screening should include genes with definite or strong evidence for being disease causing, such as the eight sarcomere genes *MYH7*, *MYBPC3*, *TNNI3*, *TNNT2*, *TPM1*, *MYL2*, *MYL3* and *ACTC1* [2,6–9] and usually genes associated with phenocopies (i.e., *LAMP2*, *GLA*, *PRKAG2* and *TTR*). Most genotyped-positive HCM patients carry defects in the thick-filament genes, myosin heavy chain (*MYH7*) and myosin-binding protein C (*MYBPC3*); less frequently in thin-filament genes, including cardiac troponin T (*TNNT2*) and I (*TNNI3*), a-tropomyosin (*TPM1*) and cardiac actin (*ACTC1*) [2,7–9]. Overall, around 50–60% of HCM patients carry P/LP variants in these sarcomeric genes. Furthermore, other genes have been associated with the disease, encoding proteins of the sarcomere apparatus (i.e., *MYH6*, *TNNC1*, *TTN*), of the Z-disc (i.e., *ACTN2*, *FLNC*, *DES*) or involved in calcium homeostasis pathways (i.e., *PLN*, *JPH2*) [10], but the strength of association with the disease is not as strong as for the eight sarcomeric genes [6]. HCM has an age-related penetrance and expressivity can be heterogenous and influenced by additional genetic [2,9–12] and acquired factors (e.g., hypertension, obesity, exercise, environmental factors) [2,3,13]. Data from the SHaRe Registry showed that being gene (+) might be associated with a worse outcome [5] and although the discussion about this finding is still ongoing [13], there is general agreement that patients with more than one sarcomere P/LP variant are more likely to progress to a dilated phenotype [3–5]. Finally, it has been suggested that P/LP variants in the so-called thin-filament genes (*TNNT2*, *TPM1*, *MYL2* and *MYL3*) are at higher risk of evolution toward heart failure [14,15] and possibly malignant arrhythmias [16].

Fatigue and dyspnoea during exercise can often occur in HCM patients [1,2,7,12]. The limited exercise performance has been attributed to several mechanisms, such as diastolic dysfunction, dynamic obstruction of the left ventricular outflow trait, left ventricular systolic dysfunction and chronotropic incompetence [2,7,12]. Objective assessment of functional capacity through the cardiopulmonary exercise test (CPET) may be useful in HCM patients, but its role in patients' risk stratification and management is still a matter of debate [17–21]. In our population, we specifically focused on the impact of genotype, carefully evaluated according to ACGM criteria [22], on CPET performance.

2. Materials and Methods

2.1. Study Population

The study was approved by our Ethical Committee. All patients signed an informed consent (2021_01_26_06 CARDGEN-REG approved by the Istituto Auxologico Italiano local Ethical Committee). We considered HCM patients followed up in our Cardiomyopathy Outpatient Clinic whose genetic analysis results were available. We included in this analysis patients in NYHA class I-II without a history of heart failure, already receiving a personalised optimal treatment as recommended by European Guidelines [2], and capable of performing a CPET within three months from an echocardiogram. Regarding echocardiographic data, we excluded patients with left ventricular (LV) dysfunction, i.e., LV ejection fraction less than 50% [2,12]. Moreover, due to the known influence on exercise performance of a significant left ventricular obstruction [23,24], we excluded from the study population patients showing, at rest or after Valsalva manoeuvre at echocardiography, a

clinically significant gradient (see below). All clinical data were collected and stored in an internal database.

2.2. Echocardiography

Echocardiograms were collected by an experienced echocardiography operator using a Vivid 9 GE echocardiographic system, using two-dimensional parasternal long-axis and short-axis views, two-chamber, three-chamber, and four-chamber apical views. Three consecutive cardiac cycles of each view were stored digitally (all patients were in sinus rhythm). LV hypertrophy was assessed with 2-dimensional echocardiography, and the site and extent of maximal wall thickness were identified. Maximal end-diastolic LV wall thickness (MWT) was used as the dimension of greatest magnitude at any site within the LV chamber [25,26]. Peak instantaneous LV outflow gradient was estimated with continuous wave Doppler under basal conditions and after Valsalva manoeuvre; LV outflow obstruction (LVOTO), due to mitral valve systolic anterior motion and mitral-septal contact, was identified either at rest or after Valsalva manoeuvre in the semi supine position [2–4,25,26]. Patients showing peak instantaneous outflow gradient $\geq$ 30 mmHg at rest or $\geq$50 mmHg after Valsalva manoeuvre, i.e., a relevant obstruction, were excluded from the study population. The following echocardiographic measurements were also collected according to guidelines: the left atrium indexed volume (LAVI), the LV ejection fraction with Simpson's biplane methods (LVEF, apical four-chamber and two-chamber views), the diastolic function parameters, the LV Global Longitudinal Strain (GLS, apical four/two and three-chamber view) and the mitral regurgitation grade [25,26].

2.3. Genetic Testing

Genetic testing was performed on blood samples through Next-Generation Sequencing (NGS, TruSight Cardio Sequencing kit, Illumina, including 197 genes). The process included the patient's DNA extraction, purification, amplification and fragmentation, followed by isolation and attachment to labelled beads for short-read sequencing [22]. The resulting alignment against a "reference" human genome sequence allowed the identifications of genetic variants in the patient's sample: all significant variants identified were confirmed with Sanger sequencing. Genetic variants were then evaluated according to their frequency in the general population (Genome Aggregation Database, Exome Variant Server, 1000 Genomes Project), presence or absence in human genetic variants databases, literature description, localisation and conservation, and they were finally classified according to ACMG guidelines; only pathogenic (P) and likely pathogenic (LP) variants were considered to classify a patient as gene (+) [22].

2.4. Cardiopulmonary Test

Exercise was performed on cycle ergometer, beginning with two minutes of rest, followed by two minutes of freewheeling warm-up, and then by a ramp-incremental load increase by 10 W per minute until volitional exhaustion. During the test, the patient breathed through a non-rebreathing mask connected to a metabolic cart Sensor Medics 2900 (Sensor Medics, 22705 Savi Ranch Pkwy, Yorba Linda, CA, USA) for breath by breath measurements of ventilation (VE, L/min), oxygen consumption (VO_2, L/min) and carbon dioxide production (VCO_2, L/min) [17]. A 12-lead ECG was monitored, and blood pressure and heart rate were measured every two minutes. Respiratory quotient (RER), VO_2 and VCO_2 were averaged during the last 30 s of exercise. Anaerobic threshold (AT) was calculated by the V-slope method. The VE/VCO_2 slope, relating the rate of increase in ventilation per unit increase in CO_2 production, was calculated by linear regression until the anaerobic compensation [26]. Other variables considered were the rate of increase in VO_2 relative to workload (VO_2/Work) that has been interpreted as an indicator of

cardiovascular efficiency [27], and O_2 pulse (pO_2, ml/beat), which is computed as VO_2/HR and potentially interpreted as a surrogate measure of stroke volume [28].

2.5. Statistics

Data are expressed as mean ± 1 standard deviation. We used Microsoft Excel statistic package for the analysis. Differences in continuous variables between groups were evaluated by unpaired *t*-test. Differences in prevalence between groups were analysed by χ^2 test with Yates' correction. A p value < 0.05 was considered significant.

3. Results

Out of 120 HCM patients fulfilling all inclusion criteria, 13 were excluded because they carried variants of uncertain significance (VUS).

Of the remaining 107 patients, 53 had a pathogenic/likely pathogenic variant (gene +) and 54 were genotype negative (gene −). The main mutated genes identified were *MYBPC3* (33 pts, 62%) and *MYH7* (12 pts, 23%); other genes (*TNNT2*, *TPM1* and *MYL3*) were found in seven patients (15%) (see Figure 1).

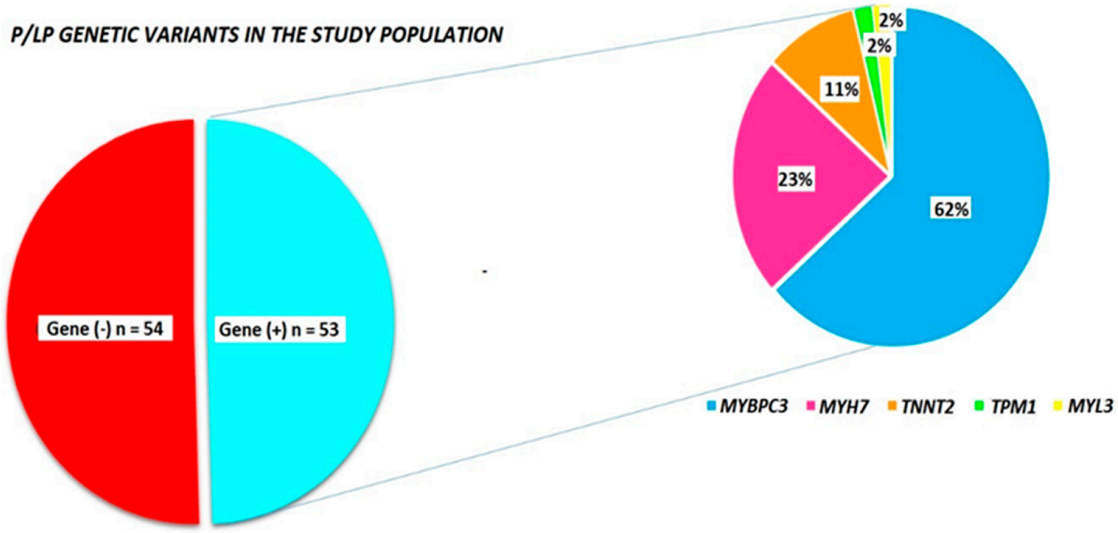

Figure 1. P/LP genetic variants distribution in the study population.

Patients' NYHA class was between I and II; none was involved in competitive sport activity.

The primary genetic analysis was focused on the eight sarcomere genes, according to guidelines [6]; however, all other genes present in the Trusight panel were evaluated and no other P/PL variants were identified.

Table 1 shows all the pathogenic/likely pathogenic variants identified, with the corresponding ACMG classification.

Table 1. Pathogenic/likely pathogenic variants identified, with the corresponding ACMG classification.

Pt Number	Gene	Type Gene	Nucleotide Change	Amino Acid Change	Class	ACMG Criteria
1	*MYBPC3*	THICK	c.G1624C	p.E542Q	P	PM3, PP1, PS3, PM2, PP3
2	*MYBPC3*	THICK	c.T3713C	p.L1238P	P	PS4, PP1, PP3, PM2
3	*MYH7*	THICK	c.G1231A	p.V411I	P	PS4, PM1, PP2, PM2, PP3
4	*MYBPC3*	THICK	c.1458-1G>A		P	PS4, PVS1, PM2
5	*MYH7*	THICK	c.G1063A	p.A355T	P	PP5, PP3, PM1, PM2
6	*MYH7*	THICK	c.C2080T	p.R694C	LP	PS4, PP1, PM1, PP2, PM2, PM5, PP3
7	*TNNT2*	thin	c.C862T	p.R288C	P	PS4, PP1, PS3, PM2, PM5, PP2

Table 1. *Cont.*

Pt Number	Gene	Type Gene	Nucleotide Change	Amino Acid Change	Class	ACMG Criteria
8	*MYBPC3*	THICK	c.G532A	p.V178M	LP	PM1, PM2, PP3 (strong)
9	*MYL3*	thin	c.G447A	p.M149I	P	PS4, PS1, PM5, PM1, PP2, PM2, PP3
10	*TNNT2*	thin	c.C418T	p.R140C	P	PS4, PP1, PS3, PM1, PP2, PM2, PP3
11	*MYH7*	THICK	c.G3346A	p.E1116K	LP	PM2, PP3 (strong), PP2, PP5
12	*MYBPC3*	THICK	c.1351+2T>C		P	PS4, PVS1, PM2
13	*MYBPC3*	THICK	c.3192dupC	p.K1065Qfs * 12	P	PS4, PP1, PVS1, PM2
	MYBPC3	THICK	c.C1112G	p.P371R	LP	PM2, PM1, PP3 (strong)
14	*TNNT2*	thin	c.A803T	p.K268I	LP	PS4, PM2, PP3, PP2
15	*MYH7*	THICK	c.G2770A	p.E924K	P	PP1, PS3, PS2, PM1, PP2, PM2, PM5, PP3
16	*TPM1*	thin	c.G172C	p.D58H	LP	PP3, PM2, PP2
17	*MYBPC3*	THICK	c.C1504T	p.R502W	P	PM3, PP1, PM2, PM5, PM1, PP3
18	*MYBPC3*	THICK	c.C1789T	p.R597W	P	PS4, PM2, PM5, PP3
19	*MYBPC3*	THICK	c.3331-1G>A		P	PS4, PVS1, PM2
20	*MYBPC3*	THICK	c.G2198T	p.R733L	LP	PM1, PM2, PM5 (strong), BP4
21	*MYH7*	THICK	c.G428A	p.R143Q	P	PS4, PP1, PM1, PP2, PM2, PM5, PP3
22	*MYBPC3*	THICK	c.C1960T	p.R654C	LP	PM1, PM2, PM5 (strong), BP5
23	*MYH7*	THICK	c.A1615C	p.M539L	P	PS4, PM1, PP2, PM2, PM5, PP3
24	*MYBPC3*	THICK	c.1458-1G>A		P	PS4, PVS1, PM2
25	*MYBPC3*	THICK	c.506-2A>C		P	PS4, PP1, PS3, PVS1, PM2
26	*TNNT2*	thin	c.C862T	p.R288C	P	PS4, PP1, PS3, PM2, PM5, PP2
27	*MYBPC3*	THICK	c.G772A	p.E258K	P	PP3, PP5, PM2, PP2
28	*MYBPC3*	THICK	c.2943_2947del	p.Q981Hfs * 67	P	PS4, PVS1, PM2
29	*MYBPC3*	THICK	c.1227-13G>A		LP	PM2, PS4, PS3, PP1, PP5
30	*MYBPC3*	THICK	c.2864_2865delCT	p.P955Rfs * 95	P	PS4, PVS1, PM2
31	*MYH7*	THICK	c.G4402C	p.E1468Q	LP	PM2, PM5, PP3, PP2
32	*MYBPC3*	THICK	c.G2459A	p.R820Q	P	PS4, PP1, PS3, PM2, PM5, PM1, PP3
33	*MYBPC3*	THICK	c.2309-2A>G		P	PS4, PP1, PVS1, PM2
34	*MYBPC3*	THICK	c.2157_2158delTG	p.C719X	P	PS4, PVS1, PM2
35	*MYBPC3*	THICK	c.2157_2158delTG	p.C719X	P	PS4, PVS1, PM2
36	*MYH7*	THICK	c.G2680A	p.E894K	P	PS4, PM1, PP2, PM2, PM5, PP3
37	*MYBPC3*	THICK	c.913_914del	p.F305Pfs * 27	P	PVS1, PP5, PM2, PS4, PP1
38	*MYBPC3*	THICK	c.G772A	p.E258K	LP	PS4, PP1, PS3, PM2, PP3
39	*MYBPC3*	THICK	c.G1828C	p.D610H	LP	PM1, PM2, PP3, PM5
40	*MYBPC3*	THICK	c.C1789T	p.R597W	P	PS4, PM2, PM5, PP3
41	*MYH7*	THICK	c.T1228C	p.Y410H	LP	PM1, PP2, PM2, PP3
42	*MYH7*	THICK	c.G428A	p.R143Q	P	PS4, PP1, PM1, PP2, PM2, PM5, PP3
43	*MYBPC3*	THICK	c.G1624C	p.E542Q	P	PM3, PP1, PS3, PM2, PP3
44	*MYH7*	THICK	c.C3133T	p.R1045C	P	PS4, PP3, PM2, PM5, PP2
	TNNT2	thin	c.A659T	p.K220M	LP	PM1, PP2, PM2, PP3
45	*MYBPC3*	THICK	c.913_914del	p.F305Pfs * 27	P	PVS1, PP5, PM2, PS4, PP1
	MYH7	THICK	c.G2012A	p.R671H	P	PS4, PM1, PM2, PP2, PM5, PP3
46	*MYBPC3*	THICK	c.2258dupT	p.K754Efs * 79	P	PS4, PVS1, PM2, PM5
47	*MYBPC3*	THICK	c.C3811T	p.R1271X	P	PM3, PS3, PVS1, PM2
48	*MYBPC3*	THICK	c.2258dupT	p.K754Efs * 79	P	PS4, PVS1, PM2, PM5
49	*MYBPC3*	THICK	c.2157_2158delTG	p.C719X	P	PS4, PVS1, PM2
50	*MYBPC3*	THICK	c.G1505A	p.R502Q	P	PS4, PM2, PM5, PM1, PP3
51	*MYBPC3*	THICK	c.339delC	p.T114Lfs * 45	LP	PVS1, PM2
52	*TNNT2*	thin	c.C418T	p.R140C	P	PS4, PP1, PS3, PM1, PP2, PM2, PP3
53	*TNNT2*	thin	c.C341T	p.A114V	LP	PM1, PP2, PM2, PM5, PP3

The symbol used (*) indicates a nonsense genetic variant.

3.1. Relevance of the Presence of P/LP Mutations

Table 2 shows patients' clinical characteristics: the two groups [gene (+) and gene (−)] were similar in terms of gender distribution, NYHA class, treatment; the only significant differences were that patients with P/LP variants were younger and less frequently affected by hypertension.

Echocardiographic characteristics are shown in Table 3: no significant between-group differences were present in LVEF, GLS, MWT, LAVI and E/e'. A mild LVOTO was found more frequently in gene (−) patients.

Table 2. Patients' clinical characteristics.

	All Pts (*n* = 107)	Gene (+) Pts (*n* = 53)	Gene (−) Pts (*n* = 54)	*p* Value
Age (years)	54 ± 16	50 ± 16	59 ± 16	0.01
Female (%)	42 (40%)	26 (42%)	20 (37%)	0.29
BMI (kg/m^2)	26.4 ± 4.4	26.2 ± 5.3	26.5 ± 3.3	0.51
Diabetes Mellitus	7 (7%)	3 (6%)	4 (7%)	0.73
Hypertension	52 (49%)	16 (30%)	32 (59%)	0.05
Active smoke	18 (17%)	11 (21%)	7 (13%)	0.56
Dyslipidemia	60 (57%)	28 (54%)	32 (59%)	0.57
ICD, primary prevention	10 (16%)	5 (9%)	6 (11%)	0.31
THERAPY (% of pts)				
β-Blockers	81 (74%)	244 (83%)	37 (69%)	0.08
Dysopiramide	5 (5%)	4 (8%)	1 (4%)	0.37
ACEi/ARB	34 (36%)	16 (37%)	18 (35%)	0.72
Ca++Blockers	11 (11%)	5 (10%)	7 (13%)	0.59
MRA	7 (8%)	5 (10%)	3 (6%)	0.63
Amiodarone	4 (4%)	2 (4%)	2 (4%)	0.90
Diuretics	5 (5%)	2 (6%)	2 (4%)	0.25

Table 3. Echocardiographic characteristics.

	All Pts (*n* = 107)	Gene (+) Pts (*n* = 53)	Gene (−) Pts (*n* = 54)	*p* Value
Maximum wall thickness (mm)	19 ± 5	19 ± 5	18 ± 4	0.21
Indexed LA Volume (mL/m^2)	46 ± 16	49 ± 19	43 ± 12	0.04
LVEF (%)	64 ± 8	63 ± 9	64 ± 8	0.73
E/e'	11.1 ± 5.0	11.0 ± 4.5	11.3 ± 5.2	0.55
GLS (%)	−15.7 ± 4.1	−16.6 ± 4.4	−15.0 ± 3.7	0.09
Mitral regurgitation, mild to moderate	81 (76%)	43 (83%)	38 (70%)	0.18
LVTO (rest or Valsalva manoeuvre)	25 (23%)	8 (15%)	17 (31%)	0.08

LA = left atrium; LVEF = left ventricular ejection fraction; GLS = global longitudinal strain; LVTO = left ventricular outflow trait obstruction.

Table 4 shows CPET data.

Table 4. Cardiopulmonary test data.

	All Pts (*n* = 107)	Gene (+) Pts (*n* = 53)	Gene (−) Pts (*n* = 54)	*p* Value
Peak RER	1.11 ± 0.15	1.12 ± 0.18	1.10 ± 0.10	0.39
Peak VO_2 (mL/kg/min)	20.5 ± 7.5	20.1 ± 7.5	20.3 ± 7.0	0.94
Peak VO_2 (% predicted)	77.0 ± 19.5	73.0 ± 16.8	80.0 ± 21.5	0.05
AT VO_2 (% VO_2 max) predicted)	52 ± 19	50 ± 21	52 ± 18	0.59
O_2 pulse peak (mL/beat)	13.1 ± 3.7	13.0 ± 3.2	13.3 ± 4.1	0.25
O_2 pulse peak (% predicted)	99.0 ± 21.4	96.5 ± 17.9	103.0 ± 23.7	0.03

Table 4. *Cont.*

	All Pts (n = 107)	Gene (+) Pts (n = 53)	Gene (−) Pts (n = 54)	p Value
VO_2/Work [(mL/kg/min)]/Watts	9.7 ± 2.3	9.4 ± 1.8	10.0 ± 2.5	0.16
VE/VCO_2 slope	31.2 ± 6.3	32.1 ± 7.0	30.7 ± 5.5	0.37
P(ET)CO_2 (mmHg)	32.0 ± 5.2	31.2 ± 6.6	33.5 ± 4.6	0.06

All patients performed a maximal volitional effort without between-group differences in RER. The overall exercise performance expressed as age-adjusted peakVO_2 and O_2 pulse [16–22] was slightly below the lower range of normality; in particular, significantly lower age-adjusted peak VO_2 and O_2 pulse were observed in gene (+) patients, i.e., those carrying a P/LP variant, than in gene (−) patients (Figure 2).

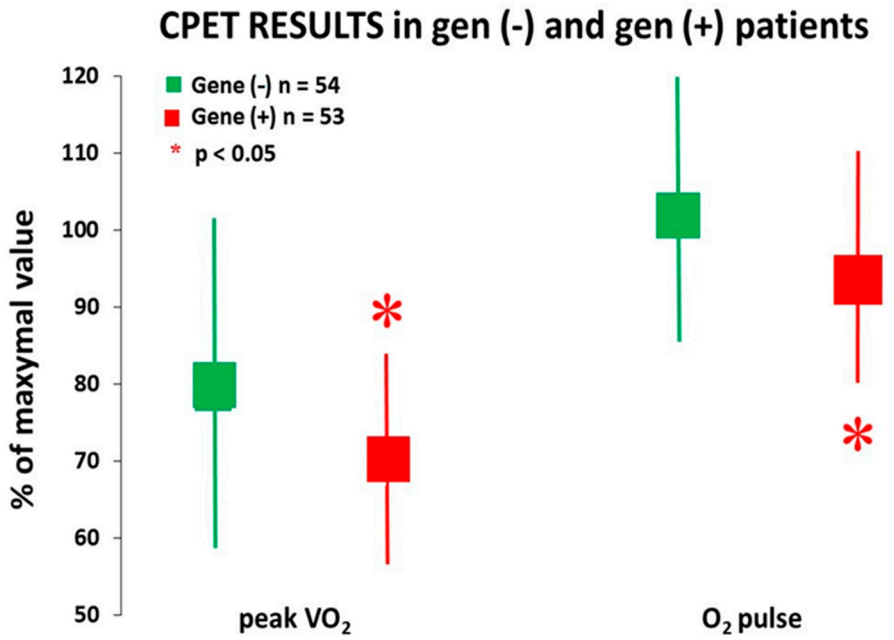

Figure 2. Peak VO_2 and O_2 pulse (as % of that predicted for age [16,17]) were significantly lower in gene (+) patients. See text for discussion.

Moreover, a moderate to severe reduction in performance (≤65% of the age-adjusted peak VO_2) [16,17] was observed in 47% (25/53 pts) of gene (+) and only in 20% (11/54 pts) of gene (−) patients ($p < 0.05$, χ^2 test).

3.2. Relevance of Different Pathogenetic P/LP Mutations

This analysis was specifically carried out in the gene (+) group: we compared patients with P/LP variants in the *thick-filament genes* [myosin heavy chain *MYH7* and myosin-binding protein C *MYBPC3* (gene + THICK), n = 45] and those in the thin-filament genes [*TNNT2*, *TPM1* and *MYL3* (gene + Thin), n = 7]. One patient, who carried P/LP variants on both *MYBPC3* and *TNNT2* genes, was excluded from this analysis; thus, we present data on 52 patients. No differences in the baseline characteristics were found according to the genes involved, as shown in Table 5.

Table 5. Baseline characteristics of the gene (+) populations.

	All Gene (+) Pts (*n* = 52)	**Gene (+THICK) Pts (*n* = 45)**	**Gene (+Thin) Pts (*n* = 7)**	***p* Value**
Age (years)	50.1 ± 14.6	49.4 ± 15.5	51.2 ± 11.7	0.71
Female (%)	22 (42%)	20 (44%)	2 (28%)	0.21
BMI (kg/m^2)	26.3 ± 5.5	26.0 ± 5.2	27.1 ± 2.9	0.81
Diabetes Mellitus	3 (6%)	2 (4%)	1 (14%)	0.38
Hypertension	20 (38%)	17 (39%)	3 (43%)	0.95
Active smoke	11 (21%)	8 (18%)	3 (43%)	0.05
Dyslipidemia	28 (54%)	23 (52%)	5 (63%)	0.59
ICD, primary prevention	7 (13%)	6 (14%)	1 (13%)	0.93
THERAPY (% of pts)				
β-Blockers	42 (81%)	35 (78%)	7 (100%)	0.22
Dysopiramide	4 (8%)	4 (9%)	none	-
ACEi/ARB	20 (38%)	15 (33%)	5 (71%)	0.11
Ca++Blockers	5 (10%)	3 (7%)	2 (25%)	0.11
MRA	5 (10%)	3 (7%)	2 (9%)	0.11
Amiodarone	2 (4%)	2 (5%)	none	-
Diuretics	3 (6%)	2 (5%)	1 (13%)	0.37

At echocardiography (Table 6), the maximum wall thickness was lower in gene (+Thin) patients compared to those gene (+THICK).

Table 6. Echocardiographic characteristics of the gene (+) populations.

	Gene (+) Pts (*n* = 52)	**Gene (+Thick) Pts (*n* = 45)**	**Gene (+Thin) Pts (*n* = 7)**	***p* Value**
Maximum wall thickness (mm)	19.7 ± 5.1	20.3 ± 5.1	16.5 ± 3.4	0.04
Indexed LA Volume (mL/m^2)	48.1 ± 15.4	47.5 ± 15.7	51.5 ± 14.3	0.50
LVEF (%)	63.3 ± 8.6	63.9 ± 7.8	60.0 ± 12.2	0.24
E/e'	10.9 ± 4.7	10.8 ± 4.8	11.38 ± 4.0	0.74
GLS (%)	−15.92 ± 4.5	−16.32 ± 4.6	−12.8 ± 3.3	0.15
Mitral regurgitation, mild to mild-moderate	41 (79%)	35 (78%)	6 (86%)	0.31
Mild LVTO at rest or after Valsalva manoeuvre	4 (8%)	4 (9%)	none	0.81

LA = left atrium; LVEF = left ventricular ejection fraction; GLS = global longitudinal strain; LVTO = left ventricular outflow trait obstruction.

Finally, when gene (+Thin) and gene (+THICK) were considered separately, patients with thin-filament gene variants showed a significantly worse exercise performance: not only age-adjusted peak VO_2 and O_2 pulse were lower, but also VO_2/Work was reduced compared to that observed in the group of gene (+THICK) patients (Table 7). Moreover, the moderate to severe reduction in CPET performance (less than 65% of the age-adjusted peak VO_2) mentioned before was present in the majority of gene (+Thin) patients (6 out of 7 patients, i.e., 86%) and only in 12 out of 45 (+THICK) patients (27%) ($p < 0.05$, χ^2 test).

Table 7. Cardiopulmonary test data in the gene (+) populations.

	All Gene (+) Pts (n = 52)	Gene (+THICK) Pts (n = 45)	Gene (+Thin) Pts (n = 7)	p Value
Peak RER	1.11 ± 0.12	1.12 ± 0.11	1.10 ± 0.16	0.21
Peak VO_2 (mL/kg/min)	20.1 ± 8	20.9 ± 7.6	16.3 ± 2.7	0.12
Peak VO_2 (% predicted)	70.5 ± 18.3	74.2 ± 15.6	58.6 ± 10.8	0.01
AT VO_2 (% predicted)	49.7 ± 21.0	49.8 ± 15.3	40.9 ± 10.9	0.15
O_2 pulse peak (mL/beat)	12.8 ± 3.56	13.4 ± 9.9	11.2 ± 2.3	0.60
O_2 pulse peak (% predicted)	94.5 ± 11.5	97.8 ± 17.0	82.2± 9.9	0.02
VO_2/Work [(mL/kg/min)]/Watts	9.3 ± 1.8	9.5 ± 1.6	8.3 ± 1.3	0.05
VE/VCO_2 slope	31.3 ± 6.9	31.7 ± 6.5	30.6 ± 8.2	0.96
P(ET)CO_2 (mmHg)	31.6 ± 7.7	32.0 ± 7.9	30.9 ± 9.2	0.61

4. Discussion

This single-centre, retrospective study presents data from consecutive HCM patients on optimal medical treatment followed up in our referral outpatients' clinic, who were asymptomatic or only slightly symptomatic for exertional dyspnoea. As a clinical strategy of our centre, they all performed CPET in addition to the recommended echocardiogram and genetic screening [2,12]. Indeed, in the last 10 years, substantial information has been collected suggesting that the cardiopulmonary test not only clarifies the pathophysiology of HCM, but also offers a prognostic insight on the progression to heart failure [19–21] or to the occurrence of malignant arrhythmias [18]. Surprisingly, despite its availability in many centres, its potential usefulness and its small cost, EU and US guidelines do not recommend CPET in the routine assessment of HCM patients, limiting its use for the evaluation of patients with severe symptoms and heart failure [2,12]. Our results suggest that CPET might add potentially useful information on the clinical status of HCM patients at an early stage of the disease.

Current guidelines recommend transthoracic 2D and Doppler echocardiography as the first line exam for the diagnosis and the early evaluation of the hypertrophic phenotype [2,12]. Standardised protocols for cross-sectional imaging from several projections are used to detect the presence, distribution and severity of hypertrophy, and to characterise the presence and severity of LVOT obstruction [25,26]. Based on echocardiographic data, for this study we preliminary excluded patients with reduced LV function and outflow tract obstruction to avoid the potential confounding evidence of these variables on exercise performance.

A positive genetic test in probands with HCM confirms the diagnosis of sarcomeric HCM, excluding the presence of phenocopies that could require a specific treatment. This finding is also important, along with thorough cascade screening, for the early identification of family-members at risk of developing the disease [2,6–12]. There is an ongoing debate on the role of genotype in risk stratification. Indeed, data from the SHaRe Registry showed that patients carrying a P/LP mutation in sarcomeric genes had a greater risk of developing arrhythmias and/or heart failure [5], but some disagreement is still present on the topic [16]. The 2022 ESC guidelines for the prevention of SCD added the genetic data as an additional risk factor that could help in the decision to implant an ICD in patients with an SCD risk score showing an intermediate risk [29]; however, the 2023 ESC guidelines on cardiomyopathies did not confirm the use of genotype in risk stratification, as the role of sarcomeric variants as a predictor of SCD, independent of SCD risk-prediction models (e.g., HCM Risk-SCD and HCM Risk-Kids), remains to be demonstrated [2]. As a matter

of fact, different P/LP sarcomeric variants may define different risk profiles: variants in thin-filament genes, compared with those in thick-filament genes, seem to be associated with an increased likelihood of advanced LV dysfunction and heart failure [7,13,16] and possibly with the development of severe ventricular arrhythmias [16].

When we analysed the results of the cardiopulmonary test keeping in mind the information obtained with echocardiography and genetic analysis, we observed some peculiar patterns of response.

On the whole, the exercise capacity observed in our patients was slightly below normal; this finding agrees with most published data [17–21]. Of note, the ventilatory response to exercise was maintained (both $VEVCO_2$ slope and $P_{(ET)}CO_2$ were normal). This observation is in contrast with previous reports on greater populations: in these studies, however, subjects with heart failure were included, and this could justify the finding of an abnormal ventilatory response [17,20]. Indeed, CPET data obtained on HCM patients without heart failure are consistent with our results [18,19,21].

In the current selected population of asymptomatic or slightly symptomatic HCM patients without signs or symptoms of heart failure and without significant LVOTO, showing only a modest functional limitation, the presence of P/LP sarcomeric variants identified subjects with a significantly poorer performance at CPET: indeed, one third of these patients showed a moderate to severe reduction in CPET performance (less than 65% of the age-adjusted peak VO_2). The worst CPET results were observed when P/LP variants were located in thin-filament genes (*TNNT2*, *TPM1* and *MYL3*): in this small group of patients, 86% showed a very poor exercise capability; in addition, the associated reduction in the VO_2/Work, compared to the group of gene (+THICK) patients, points to a more advanced impairment of cardiovascular efficiency in these patients.

These observations are in line with previous reports of a less favourable clinical outcome in patients with P/LP variants located in thin-filament genes, who more frequently show an evolution toward heart failure [13–15]. The presence of P/LP mutations (and of some of them specifically) and a slightly reduced CPET performance might thus suggest an unfavourable clinical evolution in HCM patients, regardless of similar clinical and echocardiographic characteristics.

As a pathophysiological explanation of our results, we can offer the following considerations. In HCM patients with a known genetic abnormality, mutations in β-cardiac myosin lead to a primary disease of the myocyte, causing abnormal actin–myosin interaction, increased myofilament Ca^{2+} sensitivity with an early phase of hypercontractility, altered transmembrane ion transport and adverse remodelling of the sarcoplasmic reticulum [30]. Furthermore, compared to gene-negative patients, patients with sarcomere myofilament variants have a more severe impairment of microvascular function and an increased prevalence of myocardial fibrosis [31]. These elements determine a progressive failure of energy handling and sarcolemma function, which may explain the worse exercise capability of gene (+) patients. Finally, even if hypercontractility is a shared hallmark of HCM [32], the underlying mechanisms differ between thick- and thin-filament mutations. Thick-filament HCM is primarily associated with increased ATPase activity and an elevated disordered relaxed state of myosin [33]. Conversely, thin-filament mutations initially disrupt calcium regulation: increased Ca^{2+} buffering and altered handling contribute to pathogenesis via Ca^{2+}-dependent signalling pathways [34].

So, despite genetic is not yet entered in conventionally used score for risk stratification, our study together with others already published [13–16] is providing evidence that genotype-positive patients represent a subgroup of HCM patients at higher risk. Multicentre studies will be needed to evaluate the independent predictive value of genotype, in order to support or exclude its use in risk stratification tools.

5. Conclusions

Cardiopulmonary test results in asymptomatic or slightly symptomatic patients with HCM show a reduced O_2 consumption and O_2 pulse, with an overall CPET performance slightly below normality. Noticeably, patients with P/LP mutations showed a worse exercise tolerance than gene-negative patients. Furthermore, mutations in the thin-filament genes were associated with the poorest test results.

Bearing in mind the limitations of a single-centre, retrospective study, the current results suggest that CPET should be performed in all HCM patients at their enrolment in a dedicated outpatients' clinic, as this exam could support risk stratification and clinical management with a small additional cost, that in Italy is similar to the cost of an echocardiogram.

Moreover, these data might prompt a detailed analysis of the role of pathogenetic variants on exercise performance in large multi-centre registries.

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References

1. Makavos, G.; Kairis, C.; Tselegkidi, M.E.; Karamitsos, T.; Rigopoulos, A.G.; Noutsias, M.; Ikonomidis, I. Hypertrophic cardiomyopathy: An updated review on diagnosis, prognosis, and treatment. *Heart Fail. Rev.* **2019**, *24*, 439–459. [CrossRef] [PubMed]
2. Arbelo, E.; Protonotarios, A.; Gimeno, J.R.; Arbustini, E.; Barriales-Villa, R.; Basso, C.; Bezzina, C.R.; Biagini, E.; Blom, N.A.; de Boer, R.A.; et al. 2023 ESC Guidelines for the management of cardiomyopathies. *Eur. Heart J.* **2023**, *44*, 3503–3626. [CrossRef] [PubMed]
3. Klues, H.G.; Schiffers, A.; Maron, B.J. Phenotypic spectrum and patterns of left ventricular hypertrophy in hypertrophic cardiomyopathy: Morphologic observations and significance as assessed by two-dimensional echocardiography in 600 patients. *J. Am. Coll. Cardiol.* **1995**, *26*, 1699–1708. [CrossRef] [PubMed]
4. Lopes, L.R.; Rahman, M.S.; Elliott, P.M. A systematic review and meta-analysis of genotype-phenotype associations in patients with hypertrophic cardiomyopathy caused by sarcomeric protein mutations. *Heart* **2013**, *99*, 1800–1811. [CrossRef]
5. Ho, C.Y.; Day, S.M.; Ashley, E.A.; Michels, M.; Pereira, A.C.; Jacoby, D.; Cirino, A.L.; Fox, J.C.; Lakdawala, N.K.; Ware, J.S.; et al. Genotype and Lifetime Burden of Disease in Hypertrophic Cardiomyopathy. Insights From the Sarcomeric Human Cardiomyopathy Registry (SHaRe). *Circulation* **2018**, *138*, 1387–1398. [CrossRef]

6. Wilde, A.A.M.; Semsarian, C.; Márquez, M.F.; Sepehri Shamloo, A.; Ackerman, M.J.; Ashley, E.A.; Sternick Eduardo, B.; Barajas-Martinez, H.; Behr, E.R.; Bezzina, C.R.; et al. European Heart Rhythm Association (EHRA)/Heart Rhythm Society (HRS)/Asia Pacific Heart Rhythm Society (APHRS)/Latin American Heart Rhythm Society (LAHRS) Expert Consensus Statement on the state of genetic testing for cardiac diseases. *Europace* **2022**, *24*, 1307–1367. [CrossRef]
7. Olivotto, I.; Cecchi, F.; Poggesi, C.; Yacoub, M.H. Patterns of disease progression in hypertrophic cardiomyopathy: An individualized approach to clinical staging. *Circ. Heart Fail.* **2012**, *12*, 535–546. [CrossRef]
8. Girolami, F.; Gozzini, A.; Pálinkás, E.D.; Ballerini, A.; Tomberli, A.; Baldini, K.; Marchi, A.; Zampieri, M.; Passantino, S.; Porcedda, G.; et al. Genetic Testing and Counselling in Hypertrophic Cardiomyopathy: Frequently Asked Questions. *J. Clin. Med.* **2023**, *12*, 2489. [CrossRef]
9. Harper, A.R.; Goel, A.; Grace, C.; Thomson, K.L.; Petersen, S.E.; Xu, X.; Waring, A.; Ormondroyd, E.; Kramer, C.M.; Ho, C.Y.; et al. Common genetic variants and modifiable risk factors underpin hypertrophic cardiomyopathy susceptibility and expressivity. *Nat. Genet.* **2021**, *53*, 135–142. [CrossRef] [PubMed] [PubMed Central]
10. Gerull, B.; Klaassen, S.; Brodehl, A. The Genetic Landscape of Cardiomyopathies. In *Genetic Causes of Cardiac Disease. Cardiac and Vascular Biology*; Erdmann, J., Moretti, A., Eds.; Springer: Cham, Switzerland, 2019; Volume 7. [CrossRef]
11. Maron, B.J.; Maron, M.S.; Semsarian, C. Genetics of hypertrophic cardiomyopathy after 20 years: Clinical perspectives. *J. Am. Coll. Cardiol.* **2012**, *21*, 705–715. [CrossRef]
12. Ommen, S.R.; Ho, C.Y.; Asif, I.M.; Balaji, S.; Burke, M.A.; Day, S.M.; Dearani, J.A.; Epps, K.C.; Evanovich, L.; Ferrari, V.A.; et al. 2024 AHA/ACC/AMSSM/HRS/PACES/SCMR Guideline for the Management of Hypertrophic Cardiomyopathy: A Report of the American Heart Association/American College of Cardiology Joint Committee on Clinical Practice Guidelines. *J. Am. Coll. Cardiol.* **2024**, *83*, 2324–2405. [CrossRef] [PubMed]
13. Bonaventura, J.; Rowin, E.J.; Chan, R.H.; Chin, M.T.; Puchnerova, V.; Polakova, E.; Macek, M., Jr.; Votypka, P.; Batorsky, R.; Perera, G.; et al. Relationship Between Genotype Status and Clinical Outcome in Hypertrophic Cardiomyopathy. *J. Am. Heart Assoc.* **2024**, *13*, e033565. [CrossRef] [PubMed] [PubMed Central]
14. Coppini, R.; Ho, C.Y.; Ashley, E.; Day, S.; Ferrantini, C.; Girolami, F.; Tomberli, B.; Bardi, S.; Torricelli, F.; Cecchi, F.; et al. Clinical phenotype and outcome of hypertrophic cardiomyopathy associated with thin-filament gene mutations. *J. Am. Coll. Cardiol.* **2014**, *64*, 2589–2600. [CrossRef]
15. Beltrami, M.; Fedele, E.; Fumagalli, C.; Mazzarotto, F.; Girolami, F.; Ferrantini, C.; Coppini, R.; Tofani, L.; Bertaccini, B.; Poggesi, C.; et al. Long-Term prevalence of systolic dysfunction in MYBPC3 versus MYH7-related hypertrophic cardiomyopathy. *Circ. Genom. Precis. Med.* **2023**, *16*, 363–371. [CrossRef]
16. Chumakova, O.S.; Baklanova, T.N.; Zateyshchikov, D.A. Clinical Features and Prospective Outcomes of Thin-Filament Hypertrophic Cardiomyopathy: Intrinsic Data and Comparative Insights from Other Cohorts. *J. Clin. Med.* **2025**, *14*, 866. [CrossRef] [PubMed] [PubMed Central]
17. Finocchiaro, G.; Haddad, F.; Knowles, J.W.; Caleshu, C.; Pavlovic, A.; Homburger, J.; Shmargad, Y.; Sinagra, G.; Magavern, E.; Wong, M.; et al. Cardiopulmonary responses and prognosis in hypertrophic cardiomyopathy: A potential role for comprehensive noninvasive hemodynamic assessment. *JACC Heart Fail.* **2015**, *3*, 408–418. [CrossRef]
18. Magrì, D.; Limongelli, G.; Re, F.; Agostoni, P.; Zachara, E.; Correale, M.; Mastromarino, V.; Santolamazza, C.; Casenghi, M.; Pacileo, G.; et al. Cardiopulmonary exercise test and sudden cardiac death risk in hypertrophic cardiomyopathy. *Heart* **2016**, *102*, 602–609. [CrossRef] [PubMed]
19. Magrì, D.; Santolamazza, C. Cardiopulmonary exercise test in hypertrophic cardiomyopathy. *Ann. Am. Thorac. Soc.* **2017**, *14*, S102–S109. [CrossRef]
20. Magrì, D.; Re, F.; Limongelli, G.; Agostoni, P.; Zachara, E.; Correale, M.; Mastromarino, V.; Santolamazza, C.; Casenghi, M.; Pacileo, G.; et al. Heart Failure Progression in Hypertrophic Cardiomyopathy—Possible Insights From Cardiopulmonary Exercise Testing. *Circ. J.* **2016**, *80*, 2204–2211. [CrossRef] [PubMed]
21. Magrì, D.; Mastromarino, V.; Gallo, G.; Zachara, E.; Re, F.; Agostoni, P.; Giordano, D.; Rubattu, S.; Forte, M.; Cotugno, M.; et al. Risk Stratification in Hypertrophic Cardiomyopathy. Insights from Genetic Analysis and Cardiopulmonary Exercise Testing. *J. Clin. Med.* **2020**, *9*, 1636. [CrossRef]
22. Richards, S.; Aziz, N.; Bale, S.; Bick, D.; Das, S.; Gastier-Foster, J.; Grody, W.W.; Hegde, M.; Lyon, E.; Spector, E.; et al. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet. Med.* **2015**, *17*, 405–424. [CrossRef] [PubMed]
23. Maron, M.S.; Olivotto, I.; Betocchi, S.; Casey, S.A.; Lesser, J.R.; Losi, M.A.; Cecchi, F.; Maron, B.J. Effect of left ventricular outflow tract obstruction on clinical outcome in hypertrophic cardiomyopathy. *N. Engl. J. Med.* **2003**, *348*, 295–303. [CrossRef] [PubMed]
24. Critoph, C.H.; Patel, V.; Mist, B.; Elliott, P.M. Cardiac output response and peripheral oxygen extraction during exercise among symptomatic hypertrophic cardiomyopathy patients with and without left ventricular outflow tract obstruction. *Heart* **2014**, *100*, 639–646. [CrossRef]

25. Lang, R.M.; Badano, L.P.; Mor-Avi, V.; Afilalo, J.; Armstrong, A.; Ernande, L.; Flachskampf, F.A.; Foster, E.; Goldstein, S.A.; Kuznetsova, T.; et al. Recommendations for cardiac chamber quantification by echocardiography in adults: An update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J. Am. Soc. Echocardiogr.* **2015**, *28*, 1–39.e14. [CrossRef] [PubMed]
26. Nagueh, S.F.; Phelan, D.; Abraham, T.; Armour, A.; Desai, M.Y.; Dragulescu, A.; Gilliland, Y.; Lester, S.J.; Maldonado, Y.; Mohiddin, S.; et al. Recommendations for Multimodality Cardiovascular Imaging of Patients with Hypertrophic Cardiomyopathy: An Update from the American Society of Echocardiography, in Collaboration with the American Society of Nuclear Cardiology, the Society for Cardiovascular Magnetic Resonance, and the Society of Cardiovascular Computed Tomography. *J. Am. Soc. Echocardiogr.* **2022**, *35*, 533–569. [CrossRef] [PubMed]
27. Myers, J.; Arena, R.; Cahalin, L.P.; Labate, V.; Guazzi, M. Cardiopulmonary Exercise Testing in Heart Failure. *Curr. Probl. Cardiol.* **2015**, *40*, 322–372. [CrossRef] [PubMed]
28. Stringer, W.W.; Hansen, J.E.; Wasserman, K. Cardiac output estimated noninvasively from oxygen uptake during exercise. *J. Appl. Physiol.* **1997**, *82*, 908–912. [CrossRef] [PubMed]
29. Zeppenfeld, K.; Tfelt-Hansen, J.; de Riva, M.; Winkel, B.G.; Behr, E.R.; Blom, N.A.; Charron, P.; Corrado, D.; Dagres, N.; de Chillou, C.; et al. 2022 ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death. *Eur. Heart J.* **2022**, *43*, 3997–4126. [CrossRef] [PubMed]
30. Zampieri, M.; Berteotti, M.; Ferrantini, C.; Tassetti, L.; Gabriele, M.; Tomberli, B.; Castelli, G.; Cappelli, F.; Stefàno, P.; Marchionni, N.; et al. Pathophysiology and Treatment of Hypertrophic Cardiomyopathy: New Perspectives. *Curr. Heart Fail. Rep.* **2021**, *18*, 169–179. [CrossRef]
31. Olivotto, I.; Girolami, F.; Sciagrà, R.; Ackerman, M.J.; Sotgia, B.; Bos, J.M.; Nistri, S.; Sgalambro, A.; Grifoni, C.; Torricelli, F.; et al. Microvascular function is selectively impaired in patients with hypertrophic cardiomyopathy and sarcomere myofilament gene mutations. *J. Am. Coll. Cardiol.* **2011**, *58*, 839–848. [CrossRef] [PubMed]
32. Spudich, J.A. Three perspectives on the molecular basis of hypercontractility caused by hypertrophic cardiomyopathy mutations. *Pflug. Arch. Eur. J. Physiol.* **2019**, *471*, 701–717. [CrossRef] [PubMed]
33. Robinson, P.; Liu, X.; Sparrow, A.; Patel, S.; Zhang, Y.-H.; Casadei, B.; Watkins, H.; Redwood, C. Hypertrophic cardiomyopathy mutations increase myofilament Ca^{2+} buffering, alter intracellular Ca^{2+} handling, and stimulate Ca^{2+}-dependent signaling. *J. Biol. Chem.* **2018**, *293*, 10487–10499. [CrossRef] [PubMed]
34. Keyt, L.K.; Duran, J.M.; Bui, Q.M.; Chen, C.; Miyamoto, M.I.; Enciso, J.S.; Tardiff, J.C.; Adler, E.D. Thin filament cardiomyopathies: A review of genetics, disease mechanisms, and emerging therapeutics. *Front. Cardiovasc. Med.* **2022**, *9*, 972301. [CrossRef]