SCUOLA DI DOTTORATO

UNIVERSITÀ DEGLI STUDI DI MILANO-BICOCCA

Department of

Medicine and Surgery

PhD program: Public Health Cycle: XXXVIII

Curriculum in Clinical Pathophysiology and Disease Prevention

THE INFLUENCE OF GENOTYPE ON THE CARDIOPULMONARY TEST RESPONSE IN PATIENTS AFFECTED BY HYPERTROPHIC CARDIOMYOPATHY

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ACADEMIC YEAR 2024/2025

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ABSTRACT

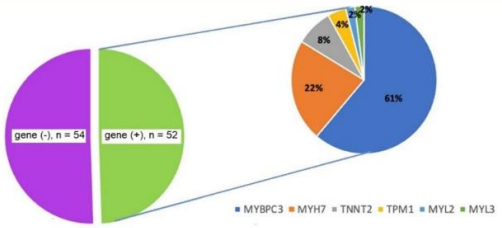
In hypertrophic cardiomyopathy (HCM), the presence of pathogenic/likely pathogenic (P/LP) disease-causing genetic variants may indicate a worse prognosis. However, there is limited data on how these genetic variants affect cardiopulmonary exercise test (CPET) performance in HCM patients. In our study, we analysed asymptomatic and slightly symptomatic HCM patients (NYHA I-II) who had both genetic analysis and CPET results available. At baseline, all participants had normal left ventricular function and severe left ventricular outflow trait obstruction was excluded. Out of 120 HCM patients, we excluded 13 who carried variants of uncertain significance; among the remaining 107 patients, 54 were genotype negative [gene (−)], and 53 had a P/LP variant in sarcomeric genes [gene (+)]. The two groups had similar NYHA class, cardiovascular risk factors and echocardiographic characteristics. However, gene (+) patients demostrated a lower peak VO2% and O2 pulse % ($p < 0.05$). Additionally, among the gene (+), those with P/LP variants in the so called “thin-filament” genes (TNNT2, TPM1 and MYL3) exhibited the poorest CPET performance. Thus, in asymptomatic or slightly symptomatic HCM patients with similar echocardiographic features, exercise tolerance is influenced by their genetic background. Specifically, gene (+) patients have reduced exercise capacity compared to gene (−) patients, and those with P/LP variants in “thin-filament” genes experience the worst performance during exercise testing.

GRAPHICAL ABSTRACT

Disease-causing genetic variants may be linked to a worse prognosis in **h**ypertrophic cardiomyopathy (HCM).

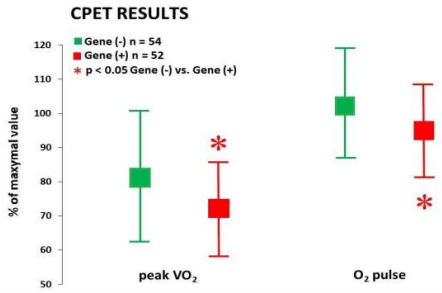
? Little is known on the effects of pathogenic/likely pathogenic (P/LP) disease-causing genetic variants on cardiopulmonary exercise test (CPET) performance in HCM patients.

We analysed data from 110 mildly symptomatic HCM patients (NYHA I-II) whose genetic analysis, baseline echocardiogram and CPET were available.
4 pts EXCLUDED because of VUS
54 pts did not show P/LP variants
52 pts had P/LP variants:
- ❑ in the *thick-filament genes* [myosin heavy chain *MYH7* and myosin binding protein C *MYBPC3* (gen + MYO) n = 44]
- ❑ in the *thin filaments genes* [*TNNT2*, *TPM1*, *MYL2*, *MYL3* (gen + THIN) n = 8].



Patients in the two groups had similar NYHA class, cardiovascular risk factors and echocardiographic characteristics.

At CPET, exercise performance expressed as age-adjusted peakVO_2 and O_2 pulse was slightly below the lower range of normality.
A significantly lower age-adjusted peak VO_2 and O_2 pulse were observed in gene (+) patients than in gene (-) patients.



In patients with P/LP variants in thin filaments genes (**gen+ THIN**), the worst exercise performance was present: age-adjusted peakVO_2 and O_2 pulse were **lower than in gen + MYO**

These preliminary data on the role of pathogenetic mutations on exercise performance need to be confirmed from large multicenter registries.

INTRODUCTION

1. Hypertrophic cardiomyopathy

1.1 Definition

Hypertrophic cardiomyopathy (HCM) is a primary heart condition characterized by an increase in left ventricular wall thickness, with or without right ventricular hypertrophy, not attributed to abnormal loading conditions [1].

In an adult, HCM is defined by an LV wall thickness ≥15 mm in any myocardial segment, lesser degrees of wall thickening (13–14 mm) require evaluation of other features including family history, genetic findings, and ECG abnormalities [2].

In children, the diagnosis of HCM requires an LV wall thickness more than 2 standard deviations greater than the predicted mean (z-score >2).

In adult first-degree relatives, the clinical diagnosis of HCM is based on the presence of LV wall thickness ≥13 mm.

1.2 Epidemiology

Studies indicate that hypertrophic cardiomyopathy (HCM) is the most common genetic heart disorder, with a prevalence ranging from approximately 1 in 200 to 1 in 500 individuals. This condition affects both sexes, occurs across all races, and can be found in people of all age groups. Morphologically, HCM is characterized by the thickening (hypertrophy) of the heart muscle (myocardium), which can present in various forms. While it frequently shows as asymmetric septal hypertrophy, it can also occur as concentric or localized hypertrophy, including involvement of the apical region. The disease may progress from compensated hypertrophy to restrictive cardiomyopathy and can

ultimately lead to end-stage heart failure due to myocardial remodeling and fibrosis. Approximately 70% of patients with HCM experience subaortic muscular obstruction to left ventricular outflow, which may be provoked or worsened by exercise or other stressors that increase myocardial contractility. An accurate clinical diagnosis requires careful consideration of both genetic and phenotypic features, underscoring the importance of integrating imaging, electrocardiographic, genetic, and clinical data. [3,4].

1.3 Aetiology

In up to 60% of adolescents and adults with HCM, the disease is an autosomal dominant trait caused by mutations in cardiac sarcomere protein genes [5, 6].

Five to ten percent of adult cases are caused by other genetic disorders including inherited metabolic and neuromuscular diseases, chromosome abnormalities and genetic syndromes [7,8].

Approximately 40% of patients HCM have nonfamilial forms, which are classified as a distinct clinical subtype. This group includes individuals (probands) who do not have an identifiable genetic cause or a family history of HCM. Nonfamilial HCM is more commonly associated with male gender, older age, a lack of asymmetric hypertrophy, and hypertension. Generally, patients in the nonfamilial HCM subgroup experience a more benign clinical course, resulting in a lower rate of adverse cardiac events compared to those with sarcomere-positive HCM. Hypertrophic cardiomyopathy can be caused by genetic disorders with variable inheritance patterns (autosomal dominant, autosomal recessive, X-linked, matrilineal).

Cardiomyopathy phenotype		AD	AR	X-linked	Matrilineal
HCM	Sarcomeric	X			
	Anderson–Fabry			X	
	Danon			X	
	TTR amyloidosis	X			
	RASopathy	X	(X)		
	Friedreich ataxia		X		
	Mitochondrial				
	Mitochondrial DNA				X
	Nuclear DNA	X	X	X	

Figure 1: Examples of inheritance patterns that should raise the suspicion of specific genetic aetiologies [2].

1.3.1 Sarcomere protein gene mutations

Mutations on sarcomere genes account for the majority of case, (60-70%) and usually have and autosomal dominant inheritance. Mutations in the genes encoding beta-myosin heavy chain (MYH7) and myosin-binding protein C (MYBPC3) account for the approximately 50% of genetic positive cases [2]. Mutations in these genes often result in more severe disease phenotypes that manifest earlier in life, particularly with specific variants.

Less commonly associated genes include TNNT2 and TNNI3 (Troponin T and I), TPM1 (α-tropomyosin), ACTC1 (α-cardiac actin), MYL2 (myosin light chain 2), MYL3 (myosin light chain 3), TRIM 63 (Tripartite motif containing 63) and CSRP3. Recent data indicate that autosomal recessive inheritance may also play a role in HCM, especially in populations where consanguinity is more common. Pathogenic variants are estimated to account for about 30%–40% of HCM cases. Pathogenic variants can alter encoded sarcomeric proteins, affecting calcium sensitivity, actomyosin contractile mechanisms, energy metabolism, and mitochondrial function in cardiomyocytes. Nevertheless, the phenotypic expression can vary significantly among

individuals. Carriers of mutations may remain asymptomatic due to incomplete penetrance or experience considerable differences in disease severity, age of onset, and clinical outcomes, often occurring within the same family. [6,9,10].

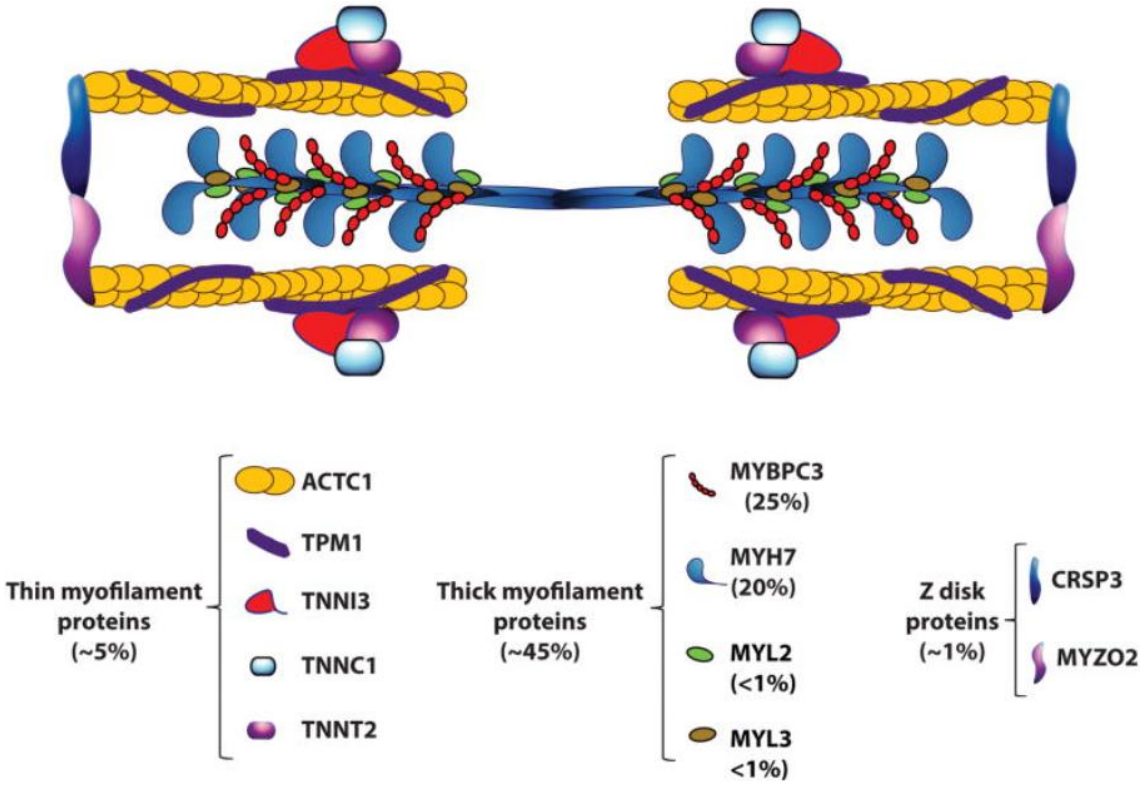


Figure 2: A schematic structure of a sarcomere composed of thick and thin filaments and Z discs [11]

Gene	Protein encoded	Frequency within genotype—positive individuals
MYBPC3	Myosin-binding protein C	40%–50%
MYH7	Beta-myosin heavy chain	35%–40%
TNNT2	Troponin T	7%–15%
TNNI3	Troponin I	5%
TPM1	Tropomyosin	3%
MYL2	Regulatory myosin light chain	1%–2%
MYL3	Essential myosin light chain	1%
ACTC1	Actin	1%
TNNC1	Troponin C	<1%
ACTN2	Alpha-actinin-2	<1%
ALPK3	Alpha-protein kinase 3	~2%
FHOD3	Formin homology 2 domain containing 3	1%–2%
CSRP3	Muscle LIM protein	<1%
TRIM63	Tripartite motif containing 63	Unknown
FLNC	Filamin C	<1%
FHL1	Four-and-a-half LIM domain protein 1	<1%
PLN	Phospholamban	<1%
JPH2	Junctophilin 2	Unknown

Figure 3: Common genetic variants in hypertrophic cardiomyopathy [12].

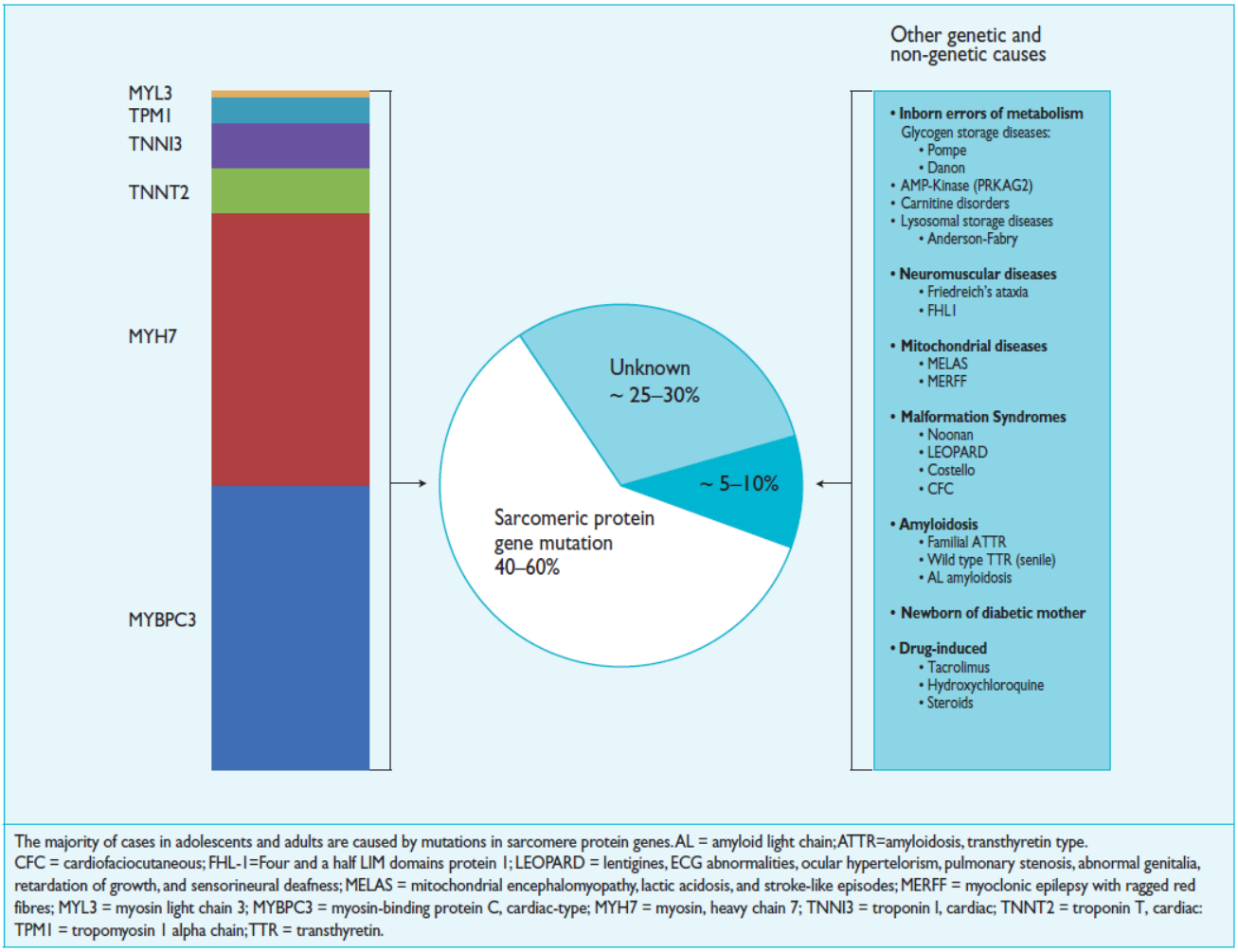
1.3.2 Other genetic and non-genetic causes

Other genes involved in the disease account for different inheritance patterns and include:

- Metabolic disorders: glycogen storage disease (Pompe, Danon), AMP-Kinase (PRKAG2), carnitine disorders, lysosomal storage disease (Anderson-Fabry)
- Neuromuscular diseases: Friedreich's ataxia, FHL-1
- Mitocondrial diseases: MELAS, MERFF
- Malformation syndromes: Noonan, Leopard, Costello, CFC
- Drug-induces: tacrolimus, hydroxychloroquine, steroids
- Infiltrative disease: hereditary transthyretin (TTR) amyloidosis and AL amyloidosis.

Then, there are idiopathic forms for which a certain cause has not yet been identified [9].

The genetics of HCM involve a combination of strong-effect mutations, polygenic modifiers, and non-genetic factors. This complexity underscores the importance of comprehensive and nuanced genetic evaluation.



The majority of cases in adolescents and adults are caused by mutations in sarcomere protein genes. AL = amyloid light chain; ATTR=amyloidosis, transthyretin type. CFC = cardiofaciocutaneous; FHL-1=Four and a half LIM domains protein 1; LEOPARD = lentigines, ECG abnormalities, ocular hypertelorism, pulmonary stenosis, abnormal genitalia, retardation of growth, and sensorineural deafness; MELAS = mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; MERFF = myoclonic epilepsy with ragged red fibres; MYL3 = myosin light chain 3; MYBPC3 = myosin-binding protein C, cardiac-type; MYH7 = myosin, heavy chain 7; TNNI3 = troponin I, cardiac; TNNT2 = troponin T, cardiac: TPM1 = tropomyosin 1 alpha chain; TTR = transthyretin.

Figure 3. Aetiology of hypertrophic cardiomyopathy [2].

1.4 Diagnosis

A comprehensive approach is critical for accurate diagnosis and to determine the underlying cause [13].

The guidelines recommend using a ‘cardiomyopathy mindset’:

- Use multimodality imaging to characterize the phenotype and identify abnormal ventricular morphology (e.g. hypertrophy, dilatation) and function (systolic/diastolic, global/regional), and detect abnormalities of tissue characterization (e.g. non-ischaemic myocardial scar).
- Use a combination of personal and family history, clinical examination, electrocardiography, and laboratory investigations to achieve an aetiological diagnosis, looking for specific signs and symptoms and laboratory markers suggestive of a specific diagnosis; the presence of ventricular and atrial arrhythmia and conduction disease to aid diagnosis, suggest specific causes, and monitor disease progression and risk stratification; and clues from the pedigree to suggest specific inheritance patterns and identify at-risk relatives.

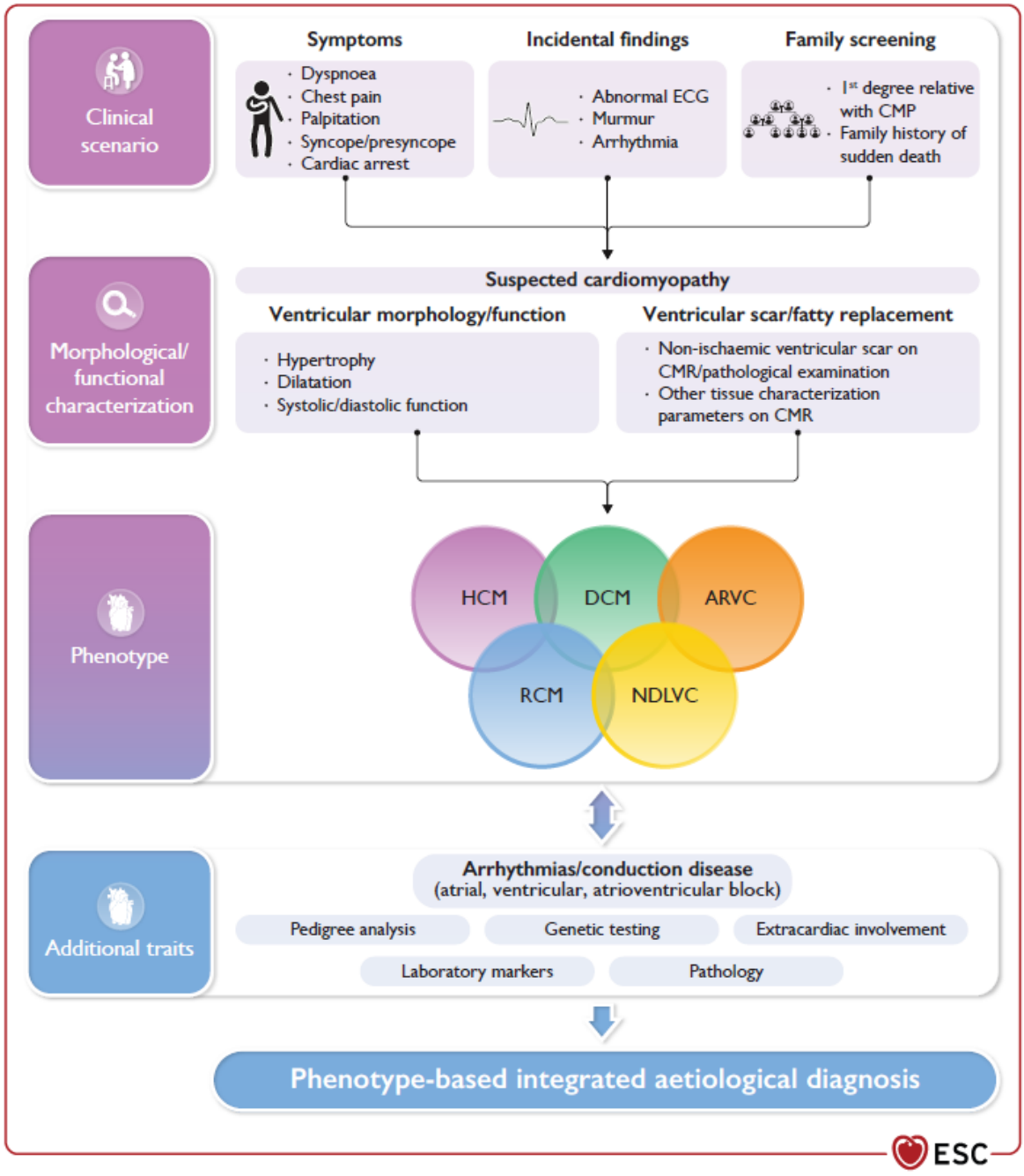


Figure 4: Clinical diagnostic workflow of cardiomyopathy [2].

1.4.1 History and physical examination

When evaluating a patient with suspected hypertrophic cardiomyopathy, it is important to consider several aspects, including:

- A three- to four-generation family pedigree; in particular, investigate premature deaths, unexplained heart failure, cardiac transplantation, pacemaker and defibrillator implants.
- Clinical history and symptoms: patients may experience dyspnoea, chest pain, palpitation, and syncope and/or pre-syncope, although many individuals complain of few, if any, symptoms.
- Physical examination is often normal but, in patients with LV outflow tract obstruction (LVOTO), a number of typical features may be identified including a rapid up-and-down stroke to the arterial pulse and an ejection systolic murmur at the left sternal edge that radiates to the right upper sternal edge and apex. The intensity of the murmur is increased by manoeuvres that reduce ventricular preload or afterload, such as standing up from the squatting position and forceful attempted exhalation against a closed airway (Valsalva manoeuvre). Most patients with LVOTO also have signs of mitral regurgitation.

1.4.2 Resting and ambulatory electrocardiography

The standard 12-lead ECG can be normal at presentation (6% of patients in referral cohort studies) but generally shows a variable combination of LVH, ST- and T-wave abnormalities, and pathological Q-waves [14].

The ECG is recommended at the first clinic visit in all individuals with known or suspected HCM and should be repeated whenever there is a change in symptoms in patients with an established diagnosis [2].

The ECG can give information on the differential diagnosis of the various forms of hypertrophic cardiomyopathy; for example, a

discrepancy between hypertrophy and low voltages is suggestive of amylodosis, whereas negative T waves in the infero-lateral derivation are suggestive of an apical form. The ECG is also a sensitive—though non-specific—early marker of disease in relatives [15].

1.4.3 Laboratory test

Routine laboratory testing aids the detection of extra-cardiac conditions that cause or exacerbate ventricular dysfunction (for example, thyroid disease, renal dysfunction and diabetes mellitus) and secondary organ dysfunction in patients with severe heart failure. High levels of brain natriuretic peptide (BNP), N-terminal pro-brain natriuretic peptide (NT-proBNP), and high-sensitivity cardiac troponin T (hs-cTnT) are associated with cardiovascular events, heart failure, and death, and may have diagnostic, prognostic, and therapeutic monitoring value [16].

There are alterations of some parameters which are indicative of certain pathologies; for example lactic acidosis, myoglobinuria, and leucocytopaenia can be suggestive of mitochondrial diseases or elevated serum levels of iron and ferritin and high transferrin saturation can suggest a diagnosis of haemochromatosis or alterations to protein electrophoresis may occur in amyloidosis.

1.4.4 Echocardiography

The non-invasive nature and widespread availability of echocardiography make it the main imaging tool, from initial diagnosis and monitoring of HCM. In most patients, hypertrophy preferentially involves the interventricular septum in the basal LV segments but often extends into the lateral wall, the posterior septum and LV apex [17].

As increased ventricular wall thickness can be found at any location (including the right ventricle), the presence, distribution, and severity of hypertrophy should be documented using a standardized protocol for cross-sectional imaging from several projections.

The following parameters must be evaluated during the echocardiography in hypertrofic cardiomyopathy:

- *Assessment of left ventricular wall thickness*: for diagnostic purposes the single most relevant parameter is the maximum LV wall thickness at any level. In patients with known or suspected HCM it is essential that all LV segments from base to apex be examined, ensuring that the wall thickness is recorded at mitral, mid-LV and apical levels.
  Meticulous imaging of the apex by parasternal and multiple apical views is required to detect apical HCM. If a segment is not visualized adequately, LV opacification—using ultrasound contrast agents and/or CMR—should be considered [18, 19]
- *Assessment of systolic function*: ejection fraction (EF) is typically normal or increased in patients with HCM. However, EF is a poor measure of LV systolic performance when hypertrophy is present [18]. Myocardial deformation imaging (speckle tracking or tissue Doppler) with global longitudinal strain is a more sensitive marker than EF to detect subtle ventricular dysfunction (e.g. in genotype-positive HCM). It is typically reduced at the site of hypertrophy (e.g. HCM) and may help discriminate between different aetiologies (e.g. apical sparing in amyloidosis) [20].
- *Assessment of diastolic function*: patients with HCM often have diastolic dysfunction and the assessment of LV filling pressures is helpful in the evaluation of symptoms and disease staging. A comprehensive evaluation of diastolic function—including Doppler

myocardial imaging, pulmonary vein flow velocities, pulmonary artery systolic pressure and LA size—is recommended as part of the routine assessment of HCM. Patients with a restrictive LV filling pattern may be at higher risk for adverse outcome, even with a preserved ejection fraction (EF) [21,22].

- *Left atrial enlargement*: the left atrium (LA) is often enlarged, and its size provides important prognostic information [23-25]. The cause of LA enlargement is multifactorial, but the most common mechanisms are systolic anterior motion (SAM)-related mitral regurgitation and elevated LV filling pressures.
- *Abnormalities of the mitral valve and left ventricular outflow tract/identification of latent obstruction*: approximately one-third of patients have resting SAM of the mitral valve leaflets that results in obstruction to the LV outflow tract, while another third have latent obstruction only during manoeuvres that change loading conditions and LV contractility; Other morphological features that contribute to LVOTO include papillary muscle abnormalities (hypertrophy, anterior and internal displacement, direct insertion into the anterior mitral valve leaflet) and mitral leaflet abnormalities such as elongation or accessory tissue [26].

Item to assess	Primary imaging modality	Comments
LV wall thickness	ECHO/CMR	• All LV segments from base to apex examined in end-diastole, preferably in the 2D short-axis view, ensuring that the wall thickness is recorded at mitral, mid-LV, and apical levels. • CMR is superior in the detection of LV apical and anterolateral hypertrophy, aneurysms,[580] and thrombi,[581] and is more sensitive in the detection of subtle markers of disease in patients with sarcomeric protein gene variants (e.g. myocardial crypts, papillary muscle abnormalities).[159,582,583]
Systolic function (global and regional)	ECHO/CMR	• Ejection fraction is a suboptimal measure of LV systolic performance when hypertrophy is present. • Doppler myocardial velocities and deformation parameters (strain and strain rate) are typically reduced at the site of hypertrophy despite a normal EF and may be abnormal before the development of increased wall thickness in genetically affected patients.
Diastolic function	ECHO	• Routine examination should include mitral inflow assessment, tissue Doppler imaging, pulmonary vein flow velocities, pulmonary artery systolic pressure, and LA size/volume.
Mitral valve	ECHO	• Assess presence and degree of SAM and mitral regurgitation. The presence of a central- or anteriorly directed jet of mitral regurgitation should raise suspicion of an intrinsic/primary mitral valve abnormality and prompt further assessment.
LVOT	ECHO	• See *Figure 12*.
LA dimensions	ECHO/CMR	• Provides important prognostic information.[365,525,584] • Most common mechanisms of LA enlargement are SAM-related mitral regurgitation and elevated LV filling pressures.

Figure 5: Imaging evaluation in hypertrophic cardiomyopathy [2].

Identification of LVOTO is important in the management of symptoms and assessment of SCD risk. Two-dimensional and Doppler echocardiography during a Valsalva manoeuvre in the sitting and semi-supine position—and then on standing if no gradient is provoked—is recommended in all patients [27,28]. Exercise stress echocardiography is recommended in *symptomatic* patients if bedside manoeuvres fail to induce LVOTO ≥50 mmHg. Pharmacological provocation with dobutamine is not advised, as it is not physiological and can be poorly tolerated.

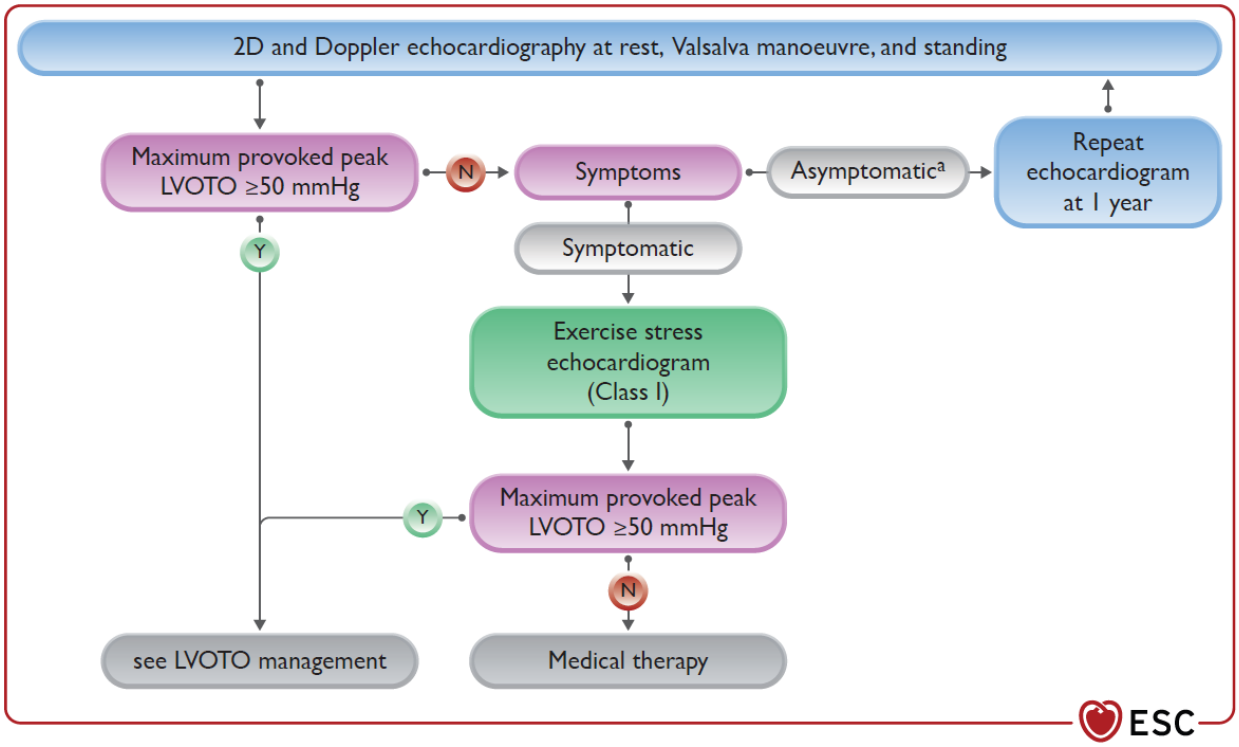


Figure 6: Protocol for the assessment and treatment of left ventricular outflow tract obstruction [2].

1.4.5 Cardiac magnetic resonance

Cardiac magnetic resonance is recommended in patients with HCM at their baseline assessment and to monitoring HCM. It provides detailed information on cardiac morphology, ventricular function and myocardial tissue characteristics. It can be particularly helpful in patients with suspected apical or lateral wall hypertrophy or LV apical aneurysm [29].

CMR may help distinguishing the causes of HCM through the distribution and severity of interstitial expansion and fibrosis distribution [30-33]. The absence of fibrosis may be helpful in differentiating HCM from physiological adaptation in athletes, but LGE may be absent in people with HCM, particularly young people and those with mild disease. As such, a

careful evaluation of ancillary abnormalities may help in the differential diagnosis.

The following parameters must be evaluated:

- *Assessment of ventricular morphology and function*: in patients with good echocardiographic images, CMR provides similar information on ventricular function and morphology, but it is helpful in establishing the diagnosis of HCM in patients with poor acoustic windows or when some LV regions are poorly visualized— such as the anterolateral wall, the LV apex and the right ventricle [34-37]. Cardiovascular magnetic resonance imaging is superior to standard 2D echocardiography in the detection of LV apical and anterolateral hypertrophy, aneurysms and thrombi, and is more sensitive in the detection of subtle markers of disease, such as myocardial crypts and papillary muscle abnormalities in patients with sarcomeric protein gene mutations [38-41]
- *Myocardial fibrosis*: Late gadolinium enhancement (LGE) is present in 65% of patients (range 33–84%), typically in a patchy mid-wall pattern in areas of hypertrophy and at the anterior and posterior RV insertion points and it's unusual in non-hypertrophied segments except in advanced stages of disease, when full-thickness LGE in association with wall thinning is common [42,43].

  The pooled data support a relationship between LGE and cardiovascular mortality, heart failure death and all-cause death, but show only a trend towards an increased risk of SCD. Late gadolinium enhancement is associated with NSVT on Holter monitoring [44-46]. But there is a debate about whether LGE is really associated with an increased arrhythmic risk; indeed LGE is present as a risk factor in

the risk stratification proposed by the American guidelines but not in the European risk score.

Moreover, with the introduction of T1 mapping, diffuse myocardial processes can be detected and quantified. This has added a new dimension to the understanding and assessment of various myocardial diseases. T1 mapping promises to detect early disease, quantify disease severity and provide prognostic insights into certain conditions. This provides an intrinsic signal from both the myocytes and the interstitium. Our current understanding is that T1 is prolonged with fibrosis, edema and amyloid (an higher native T1 is patognomic for amyloidosis) [29].

Myocardial fibrosis/LGE	CMR	• The distribution and severity of interstitial expansion can suggest specific diagnoses. Anderson–Fabry disease is characterized by a reduction in non-contrast T1 signal and the presence of posterolateral LGE.[134,155] In cardiac amyloidosis, there is often global, subendocardial or segmental LGE and a highly specific pattern of myocardial and blood-pool gadolinium kinetics caused by similar myocardial and blood T1 signals.[585,586]

Figure 7: Imaging evaluation in hypertrophic cardiomyopathy [2].

1.4.6 Genetic testing and family screening

In about half of cases, HCM is inherited as a Mendelian genetic trait. In such cases, the inheritance is primarily autosomal dominant, i.e. with a 50% risk of transmission to offspring. Apparently sporadic cases can have a monogenic cause, either because of incomplete penetrance of a variant inherited from a parent or due to de novo variants that were not carried by the parents or, less commonly, due to autosomal recessive inheritance. In those who undergo genetic testing, ~40–60% will have a single variant identified as the cause of their disease, although this is influenced by the cohort studied [42]. The likelihood of finding a causal variant is highest in young patients with familial disease and lowest in older patients and individuals with non-classical features.

In families in whom a disease-causing genetic variant has been identified, cascade genetic testing should be offered. In particular, predictive genetic testing in related children is recommended in those aged >10–12 years [47].

Individuals found not to carry the familial variant and who do not have a clinical phenotype can usually be discharged, with advice to seek re-assessment if they develop symptoms or when new clinically relevant data emerge in the family. Those relatives harbouring the familial genetic variant(s) should undergo regular clinical evaluation with ECG, multimodality cardiac imaging, and additional investigations (e.g. Holter monitoring) guided by age, family phenotype, and genotype.

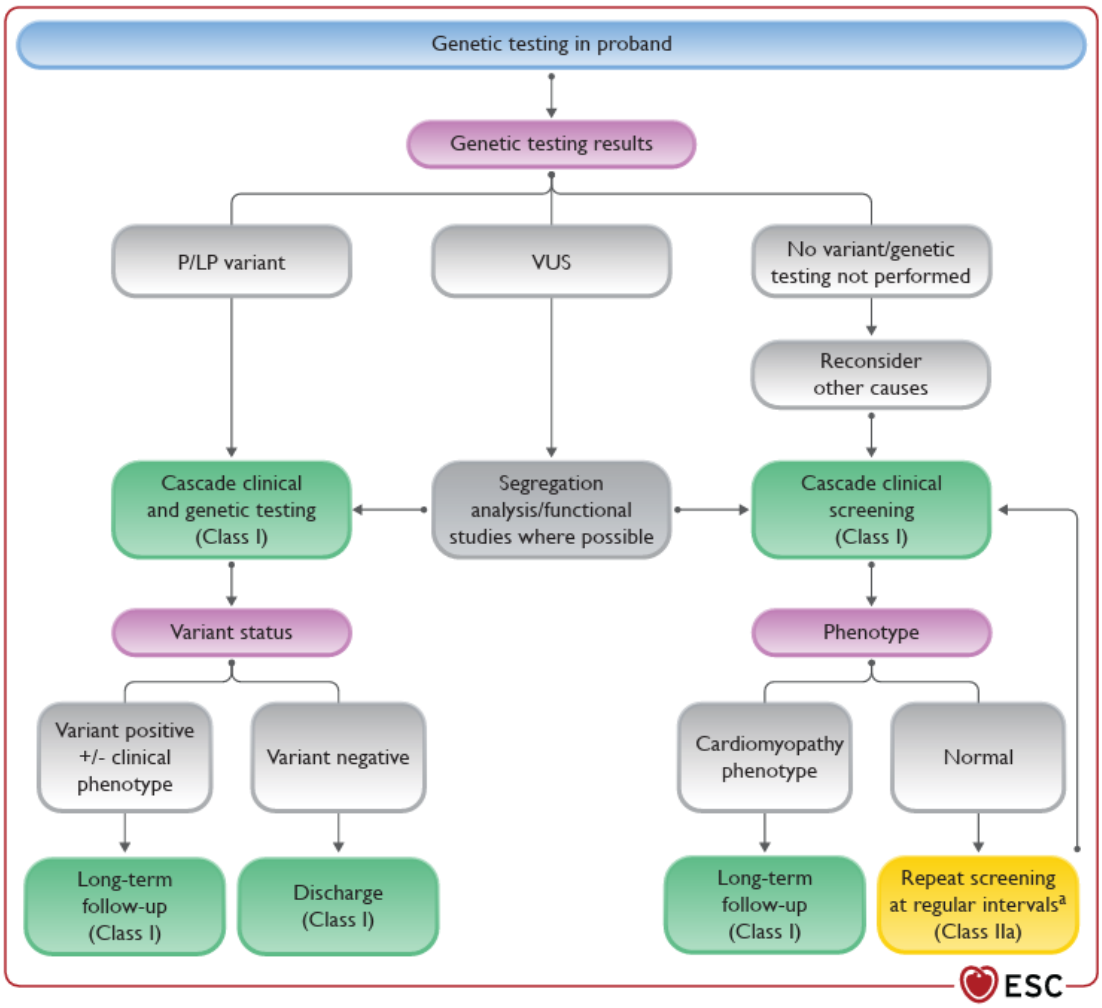


Figure 8: Algorithm for the approach to family screening and follow-up of family members [2].

1.5 Therapy

Most people with HCM are asymptomatic and have a normal lifespan but some develop symptoms, often many years after the appearance of ECG or echocardiographic evidence of LVH. Systematic two-dimensional (2D) and Doppler echocardiography, resting and 48 hr ambulatory ECG monitoring, and exercise testing are usually sufficient to determine the most likely cause of symptoms. Additional investigations (e.g. coronary CT scanning or coronary angiography, cardio-pulmonary exercise testing [CPET], electrophysiological study, loop recorder implantation) should be considered to investigate specific symptoms of chest pain, syncope, and palpitation, according to established clinical practice and guidelines. Specifically, there are mutations in genes encoding sarcomere proteins, such as MYH7, MYBPC3, TNNT2, TNNI3, which are associated with coronary anomalies, structural defects such as ventricular defects or ductus arteriosus [2,43-47].

Cardiac catheterization to evaluate right and left heart function and pulmonary arterial resistance, and CPET with simultaneous measurement of respiratory gases, is not a standard part of the work-up, but remains recommended in severely symptomatic patients with systolic and/or diastolic LV dysfunction when uncertainty about filling status exists, or for those being considered for heart transplantation or mechanical circulatory support [46].

1.5.1 Heart failure management

In the 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure, recommendations are generally independent from

the aetiology of heart failure and include current medical therapy, devices, and LV assist device (LVAD)/transplantation. Medical therapies for HFrEF based on randomized controlled trials (RCTs) from large cohorts, including angiotensin-converting enzyme inhibitors (ACE-I)/angiotensin receptor neprilysin inhibitors (ARNIs), beta-blockers, mineralocorticoid receptor antagonists (MRA), and sodium–glucose co-transporter 2 inhibitors (SGLT2i). Recommendations for management of HFpEF would be mainly applicable to non-obstructive HCM. If heart failure symptoms are present, loop diuretics should be given, although orthostatic hypotension may cause intolerance, and excessive fluid loss may worsen symptoms due to restriction. There is no defined role for the heart failure drugs because there are no specific studies but it is likely that they could play a role in the future.

1.5.2 Atrial arrhythmias management

Atrial fibrillation is the most common arrhythmia in all subtypes of cardiomyopathies and is associated with an increased risk of cardio-embolic events, heart failure, and death [48-50]. Risk scores for both AF and thrombosis have been proposed in HCM but this is not yet in clinical practice [51-52]. The aspects to consider are the management of:

- *Anticoagulation:* HCM is associated with a particularly increased risk of stroke. The CHA2DS2-VASc (congestive heart failure or left ventricular dysfunction, hypertension, age ≥75 [doubled], diabetes, stroke [doubled]-vascular disease, age 65– 74, sex category [female]) score has not been specifically tested in patients with cardiomyopathies and retrospective evidence suggests that it may perform suboptimally with respect to stroke prediction in HCM and ATTR amyloidosis. For this reason, although there are no RCTs

evaluating the role of anticoagulation among patients with HCM, given the high incidence of stroke, prophylactic anticoagulation is recommended in all patients with HCM and AF [53-56].

- *Rate control:* observational studies suggest that higher heart rates are associated with worse outcomes in patients with heart failure. Beta-blockers are the preferred choice in patients with cardiomyopathies given their long-established safety in the presence of LV dysfunction [55,56], non-dihydropyridine calcium channel blockers (CCBs) (verapamil or diltiazem) may only be used in patients with LVEF ≥40% [57]. Atrioventricular node ablation is also an alternative in patients with poor ventricular rate control despite medical treatment not eligible for rhythm control by catheter ablation or in patients with biventricular pacing [57]. In patients with symptomatic persistent AF (>6 months) unsuitable for AF ablation or in which AF ablation had failed, narrow QRS and at least one admission for heart failure, AV node ablation in association with CRT has been shown to be superior to rate control with pharmacological therapy.
- *Rhythm control:* maintenance of sinus rhythm is highly desirable and a rhythm control strategy is preferred, particularly in the presence of symptoms. There is a potential for proarrhythmia of class I antiarrhythmics, particularly in the presence of significant structural heart disease, so the antiarrhythmic drug–drug treatment has mostly been limited to amiodarone or sotalol. Catheter ablation of AF is a safe and superior alternative to AAD therapy for maintenance of sinus rhythm, reducing AF-related symptoms, and improving QoL, and can be considered an alternative to AAD therapy in practically any type and context of AF [57]. Maintenance is achieved in up to two-thirds of patients, although repeat procedures or continuation of

antiarrhythmic medications are often necessary. Patients with cardiomyopathies may have a higher risk of AF recurrence, particularly in the presence of atrial remodelling/dilatation [58-64].

1.5.3 Management of left ventricular outflow tract obstruction

All patients with LVOTO should avoid dehydration and excess alcohol consumption, and weight loss should be encouraged. Arterial and venous dilators, including nitrates and phosphodiesterase type 5 inhibitors, can exacerbate LVOTO and should be avoided if possible. New-onset or poorly controlled AF can exacerbate symptoms caused by LVOTO and should be managed by prompt restoration of sinus rhythm or ventricular rate control [65,66]. Patients with symptomatic LVOTO have been treated initially with non-vasodilating beta- blockers titrated to the maximum tolerated dose, but there are very few studies comparing individual beta-blockers. If beta-blockers alone are ineffective, disopyramide, titrated up to a maximum tolerated dose (usually 400–600 mg/day), may be added [67-69]. Dose-limiting anticholinergic side effects include dry eyes and mouth, urinary hesitancy or retention, and constipation. The QTc interval should be monitored during dose up-titration and the dose reduced if it exceeds 500 ms. Disopyramide should be avoided in patients with glaucoma, in men with prostatism, and in patients taking other drugs that prolong the QT interval, such as amiodarone and sotalol. Disopyramide may be used in combination with verapamil [68]. Verapamil (starting dose 40 mg three times daily to maximum 480 mg daily) can be used when beta-blockers are contraindicated or ineffective but, based on limited data, should be used

cautiously in patients with severe obstruction (≥100 mmHg) or elevated pulmonary artery systolic pressures, as it may provoke pulmonary oedema [70]. Another drugs useful to management of LVOTO are cardiac myosin ATPase inhibitors (Mavacamten) that is a first-in-class cardiac myosin adenosine triphosphatase (ATPase) inhibitor that acts by reducing actin–myosin cross-bridge formation, thereby reducing contractility and improving myocardial energetics; in the recently published Clinical Study to Evaluate Mavacamten in Adults with Symptomatic Obstructive Hypertrophic Cardiomyopathy (EXPLORER-HCM) trial, mavacamten reduced the left ventricular outflow tract (LVOT) gradient and improved exercise capacity compared with placebo in patients with HCM and symptomatic LVOTO (NYHA II–III and EF >55%) [72]. Another cardiac myosin ATPase inhibitors that has proven effective in hypertrophic obstructive cardiomyopathy is Aficamten (SEQUOIA-HCM study).

The invasive treatment of left ventricular outflow tract (surgery and alcohol septal ablation) to reduce LVOTO should be considered in patients with a LVOTO gradient ≥50 mmHg, severe symptoms (NYHA functional class III–IV), and/or exertional or unexplained recurrent syncope in spite of maximally tolerated drug therapy. Invasive therapy may also be considered in patients with mild symptoms (NYHA class II) refractory to medical therapy who have a resting or maximum provoked gradient of ≥50 mmHg (exercise or Valsalva) and moderate-to-severe systolic anterior motion-related mitral regurgitation, AF, or moderate-to-severe left atrial dilatation in expert centres with demonstrable low procedural complication rates [72]. The most commonly performed surgical procedure to treat LVOTO is ventricular

septal myectomy, in which a rectangular trough that extends distally to beyond the point of the mitral leaflet–septal contact is created in the basal septum below the aortic valve [73]. This abolishes or substantially reduces LV outflow tract gradients in over 90% of cases, reduces systolic anterior motion-related mitral regurgitation, and improves exercise capacity and symptoms. The main surgical complications are AV nodal block, left bundle branch block (LBBB), ventricular septal defect, and aortic regurgitation. Alcohol septal ablation can be considered in experienced centres, selective injection of alcohol into a septal perforator artery to create a localized septal scar has outcomes similar to surgery in terms of gradient reduction, symptom improvement, and exercise capacity, including in younger adults [74-81]. In the end, permanent AV sequential pacing with short AV interval may be considered in symptomatic adult patients who are unsuitable for— or unwilling to consider—other invasive septal reduction therapies, and in patients who have other pacing indications. Several long-term observational studies have reported reductions in LV outflow tract gradients and variable improvement in symptoms and quality of life [82-86]. A recent Cochrane review concluded that the data on the benefits of pacing are based on physiological measures and lack information on clinically relevant end-points [87].

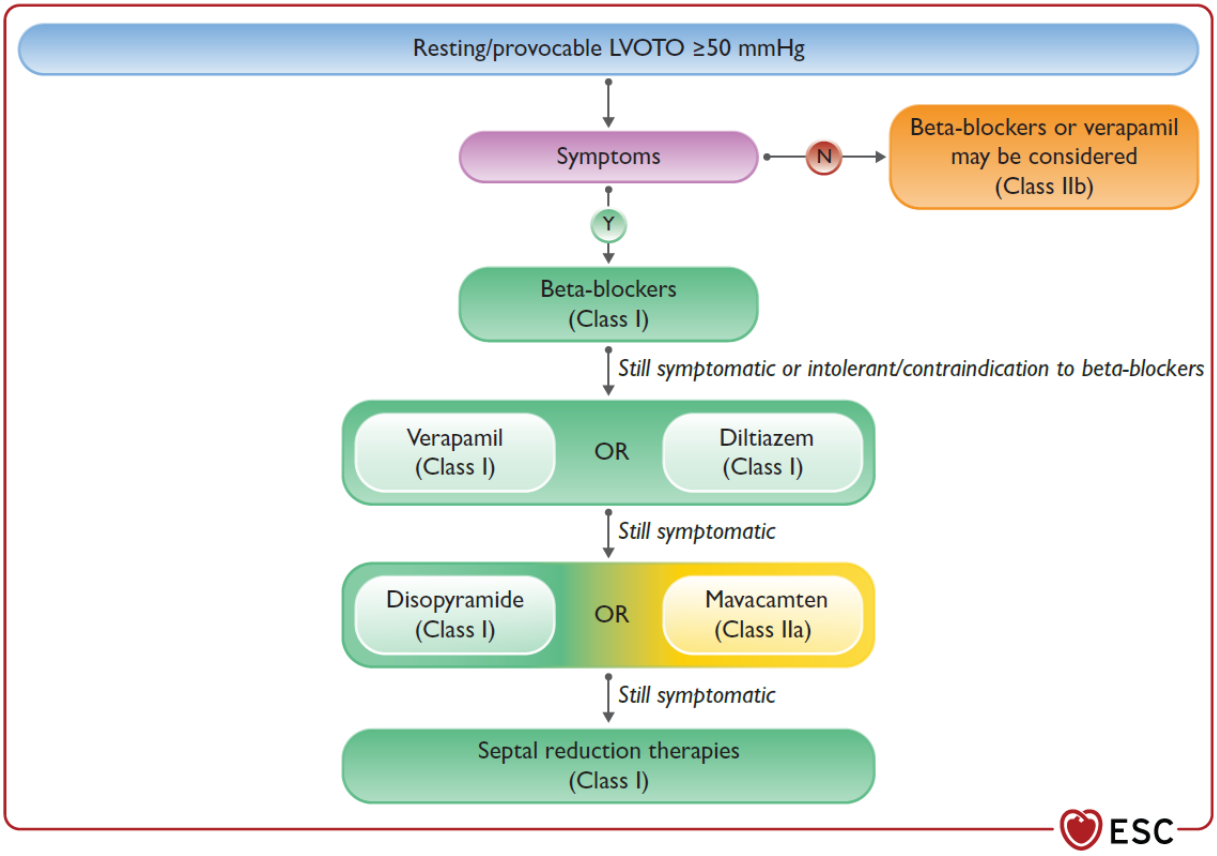


Figure 9: Flow chart on the management of left ventricular outflow tract obstruction [2].

1.5.4 Sudden cardiac death prevention

Most contemporary series of adult patients with HCM report an annual incidence for cardiovascular death of 1–2%, with SCD, heart failure, and thrombo-embolism being the main causes of death [88]. The most commonly recorded fatal arrhythmic event is spontaneous ventricular fibrillation (VF), but asystole, AV block, and pulseless electrical activity are described [89-94].

Estimation of SCD risk is an integral part of clinical management. Clinical features that are associated with an increased SCD risk are reported in the table below.

Risk factor	Comment
Age	-The effect of age on SCD has been examined in a number of studies and two have shown a significant association, with an increased risk of SCD in younger patients. -Some risk factors appear to be more important in younger patients, most notably NSVT, severe LVH, and unexplained syncope. -Sudden cardiac death is very rare below the age of 6 years and there are some data to suggest a peak of SCD in childhood HCM between 9 and 15 years; however, the association between age at diagnosis and SCD risk in childhood HCM remains unclear.
NSVT	-NSVT (defined as ≥3 consecutive ventricular beats at ≥120 b.p.m. lasting <30 s) occurs in 20–30% of patients during ambulatory ECG monitoring and is an independent predictor of SCD. -There is no evidence that the frequency, duration, or rate of NSVT influences the risk of SCD. -NSVT occurring during or immediately following exercise is very rare, but may be associated with a high risk of SCD.
Maximum LV wall thickness	-The severity and extent of LVH measured by TTE are associated with the risk of SCD. -Several studies have shown the greatest risk of SCD in patients with a maximum wall thickness of ≥30 mm; however, there are few data in patients with extreme hypertrophy (≥35 mm)
Family history of sudden cardiac death at a young age	-While definitions vary,a family history of SCD is usually considered clinically significant when one or more first-degree relatives have died suddenly aged <40 years with or without a diagnosis of HCM, or when SCD has occurred in a first-degree relative at any age with an established diagnosis of HCM. -Family history of SCD does not appear to be an independent risk factor for SCD in childhood HCM. This may be due to a higher prevalence of *de novo* variants in childhood HCM, the inclusion of non-sarcomeric disease, and/or under-reporting of family history in paediatric cohorts.
Syncope	-Syncope is common in patients with HCM but is challenging to assess, as it has multiple causes. -Non-neurocardiogenic syncope for which there is no explanation after investigation is associated with an increased risk of SCD. -Episodes within 6 months of evaluation may be more predictive of SCD.
Left atrial diameter	-Several studies have reported a positive association between LA size and SCD. There are no data on the association between SCD and LA area or volume. Measurement of LA size is also important in assessing the risk of AF
LV outflow tract obstruction	-A number of studies have reported a significant association between LVOTO and SCD risk. Several unanswered questions remain, including the prognostic importance of provocable LVOTO and the impact of treatment (medical or invasive) on SCD. -In childhood HCM, there are conflicting data on the association between LVOTO and SCD risk.

Figure 10: Major clinical features associated with an increased risk of sudden cardiac death [2].

There are no randomized trials or statistically validated prospective prediction models that can be used to guide ICD implantation in patients with HCM. Recommendations are instead based on observational, retrospective cohort studies that have determined the relationship between clinical characteristics and prognosis. The 2014 ESC Guidelines on diagnosis and management of hypertrophic cardiomyopathy recommended a risk-prediction model—HCM Risk-SCD that provides

individualized, quantitative risk estimates using an enhanced phenotypic approach [90]. This approach has since been validated in independent cohorts and a meta-analysis of available published data, relevant to the *2014 ESC Guidelines on diagnosis and management of hypertrophic cardiomyopathy* performance, for SCD prevention has shown that pooled estimates are concordant with the observed SCD risk in patients designated as high or low risk [91-94]. In the *2023 ESC Guidelines for the management of cardiomyopathies*, the Task Force maintains the principle of risk estimation using the validated HCM Risk-SCD tool as the first step in sudden death prevention in patients aged 16 years or more, and recommends the use of a validated risk score (e.g. HCM Risk-Kids tool) for children and adolescents <16 years. This is in contrast to the 2020 AHA/ACC Guideline for the diagnosis and treatment of patients with hypertrophic cardiomyopathy [92], in which the tool is considered an aid to a shared decision-making process for ICD placement in patients with clinical risk markers.

Figure 11 summarizes the recommendations for primary prevention ICD implantation in HCM in each risk category.

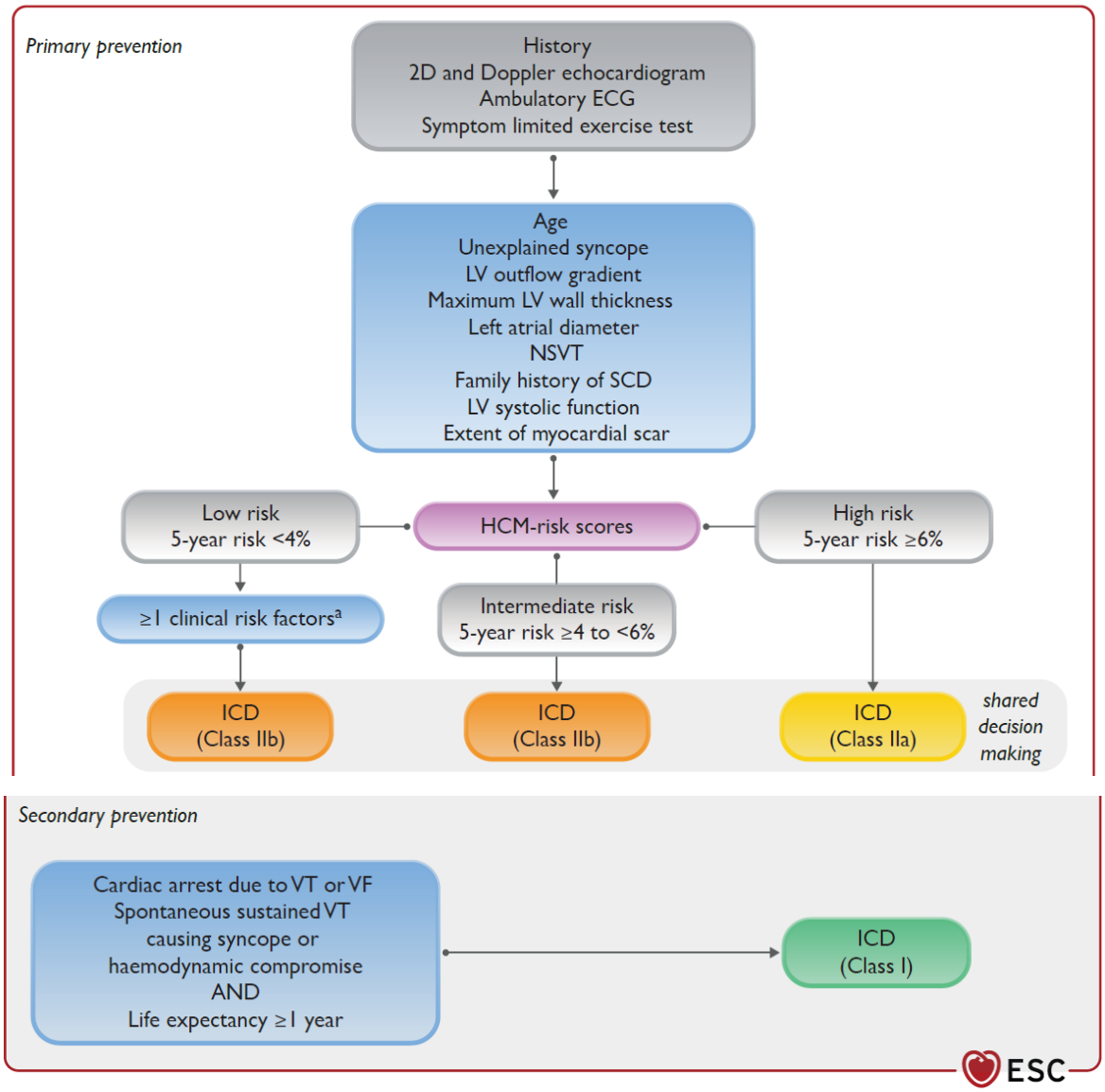
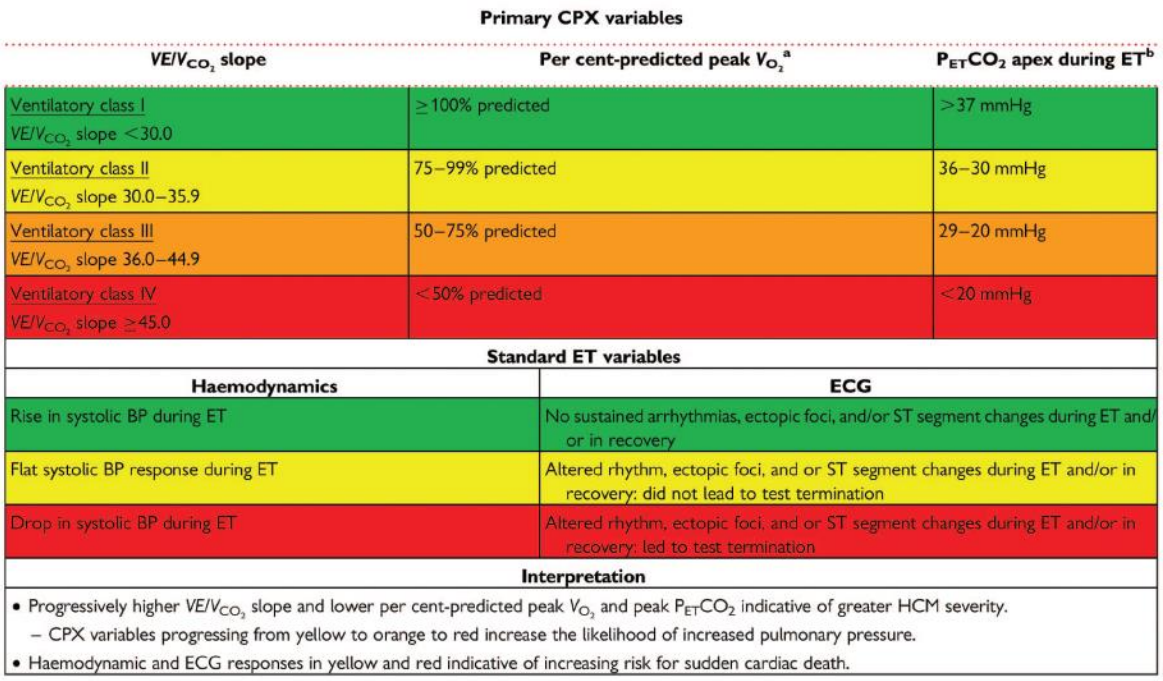


Figure 11: Flow chart for implantation of an implantable cardioverter defibrillator in patients with hypertrophic cardiomyopathy [2].

2. Cardiopulmonary test

Cardiopulmonary exercise testing (CPET) combines standard exercise testing measurements (i.e., blood pressure, ECG, and symptom assessment) with ventilatory expired gas analysis. The use of CPET provides enhanced information on the severity of the disease and its mechanism of functional limitation compared to that of standard exercise testing and stress echocardiography, which are limited due to the ECG changes and wall motion abnormalities that are common in HCM in the absence of coronary artery disease [95]. A cycle ergometer or a treadmill is an acceptable exercise modality for CPET in patients diagnosed with HCM [95]. CPET is used to quantify cardiorespiratory fitness, discover the pathophysiological mechanism underlying exercise intolerance and formulate a function based prognostic stratification [96,97]. CPET provides a detailed and comprehensive way to approach the complex pathophysiology of HCM and can be a useful tool in assessing prognosis and treatment, especially in recognizing patients with a higher risk for sudden cardiac death and HF development [98,99]. CPET is also a useful tool in differentiating HCM from other forms of LV hypertrophy, such as athletes' heart, as well as in the evaluation of athletes with a confirmed diagnosis of HCM [100]. Moreover, CPET is used to monitor therapeutic efficacy in this patient population [101]. A number of monitored and calculated CPET parameters may be helpful in targeting HCM diagnosis and assessing its risks, including but not limited to the following: blood pressure, HR and ECG changes, maximal or peak oxygen consumption (VO2), percentage of age- and sex-predicted maximal/peak VO2, ventilatory anaerobic threshold (VAT), oxygen O2 pulse (i.e., amount of oxygen extracted by tissues per heart beat), ventilatory efficiency (i.e., minute ventilation (VE)/carbon-dioxide (CO2) production slope), partial

pressure of end-tidal CO2 pressure (PETCO2), and pattern of breathing, or respiratory reserve at the end of exercise (BR) [97,99].



Primary CPX variables		
VE/V_{CO_2} slope	Per cent-predicted peak V_{O_2}[a]	$P_{ET}CO_2$ apex during ET[b]
Ventilatory class I VE/V_{CO_2} slope <30.0	≥100% predicted	>37 mmHg
Ventilatory class II VE/V_{CO_2} slope 30.0–35.9	75–99% predicted	36–30 mmHg
Ventilatory class III VE/V_{CO_2} slope 36.0–44.9	50–75% predicted	29–20 mmHg
Ventilatory class IV VE/V_{CO_2} slope ≥45.0	<50% predicted	<20 mmHg

Standard ET variables	
Haemodynamics	**ECG**
Rise in systolic BP during ET	No sustained arrhythmias, ectopic foci, and/or ST segment changes during ET and/or in recovery
Flat systolic BP response during ET	Altered rhythm, ectopic foci, and or ST segment changes during ET and/or in recovery: did not lead to test termination
Drop in systolic BP during ET	Altered rhythm, ectopic foci, and or ST segment changes during ET and/or in recovery: led to test termination

Interpretation

- Progressively higher VE/V_{CO_2} slope and lower per cent-predicted peak V_{O_2} and peak $P_{ET}CO_2$ indicative of greater HCM severity.
  - CPX variables progressing from yellow to orange to red increase the likelihood of increased pulmonary pressure.
- Haemodynamic and ECG responses in yellow and red indicative of increasing risk for sudden cardiac death.

Figure 12: Prognostic and Diagnostic Stratification for Patients With Confirmed or Suspected HCM [102].

3. Materials and Methods

3.1. 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% [2,103]. Moreover, due to the known influence on exercise performance of a significant left ventricular obstruction [104,105], 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.

3.2. 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 [106,107]. 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, 104]. Patients showing peak instantaneous outflow gradient ≥ 30 mmHg at rest or ≥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 [106,107].

3.3. 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 [108]. 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 (+) [108].

3.4. 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 (VO2, L/min) and carbon dioxide production (VCO2, L/min) [117]. A 12-lead ECG was monitored, and blood pressure and heart rate were measured every two minutes. Respiratory quotient (RER), VO2 and VCO2 were averaged during the last 30 s of exercise. Anaerobic threshold (AT) was calculated by the V-slope method. The VE/VCO2 slope, relating the rate of increase in ventilation per unit increase in CO2 production, was calculated by linear regression until the anaerobic compensation [107]. Other variables considered were the rate of increase in VO2 relative to workload (VO2/Work) that has been interpreted as an indicator of cardiovascular efficiency [127], and O2 pulse (pO2, ml/beat), which is computed as VO2/HR and potentially interpreted as a surrogate measure of stroke volume [108].

3.5. 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 $\chi 2$ test with Yates' correction. A p value < 0.05 was considered significant.

4. 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 13).

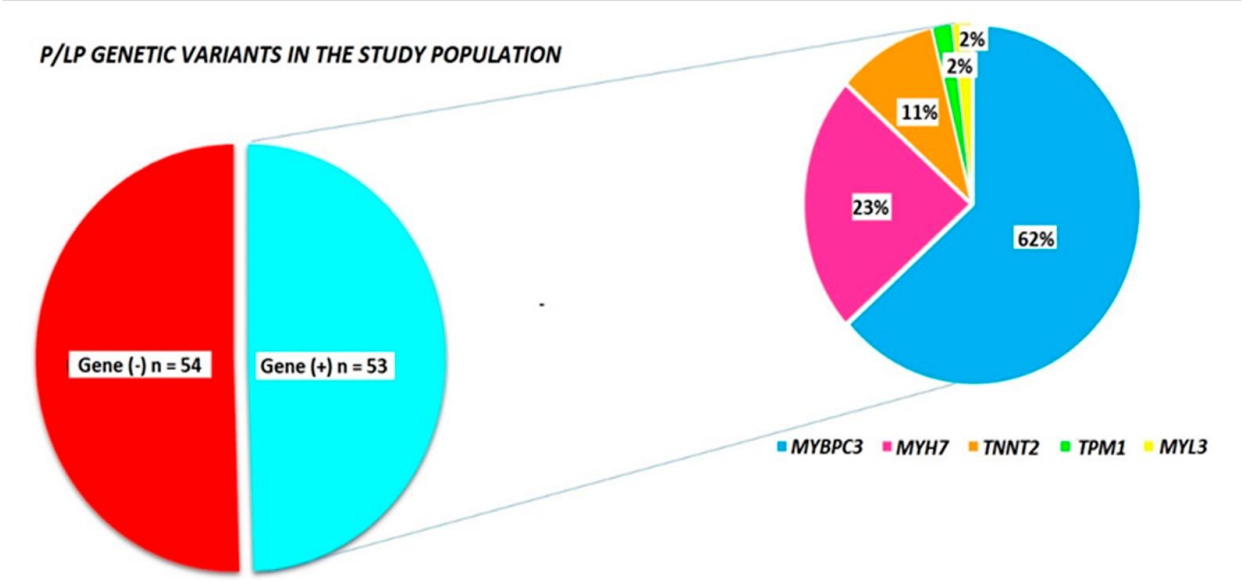


Figure 13. P/LP genetic variants distribution in the study population.

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; however, all other genes present in the Trusight panel were evaluated and no other P/PL variants were identified.

The table 1 shows all the 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
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, PI
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

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

4.1. 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.

	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 2. Patients' clinical 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 3. Echocardiographic characteristics.

Table 4 shows CPET 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
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

Table 4. Cardiopulmonary test data.

All patients performed a maximal volitional effort without between-group differences in RER. The overall exercise performance expressed as age-adjusted peakVO2 and O2 pulse [108-114] was slightly below the lower range of normality; in particular, significantly lower age-adjusted peak VO2 and O2 pulse were observed in gene (+) patients, i.e., those carrying a P/LP variant, than in gene (−) patients (Figure 14).

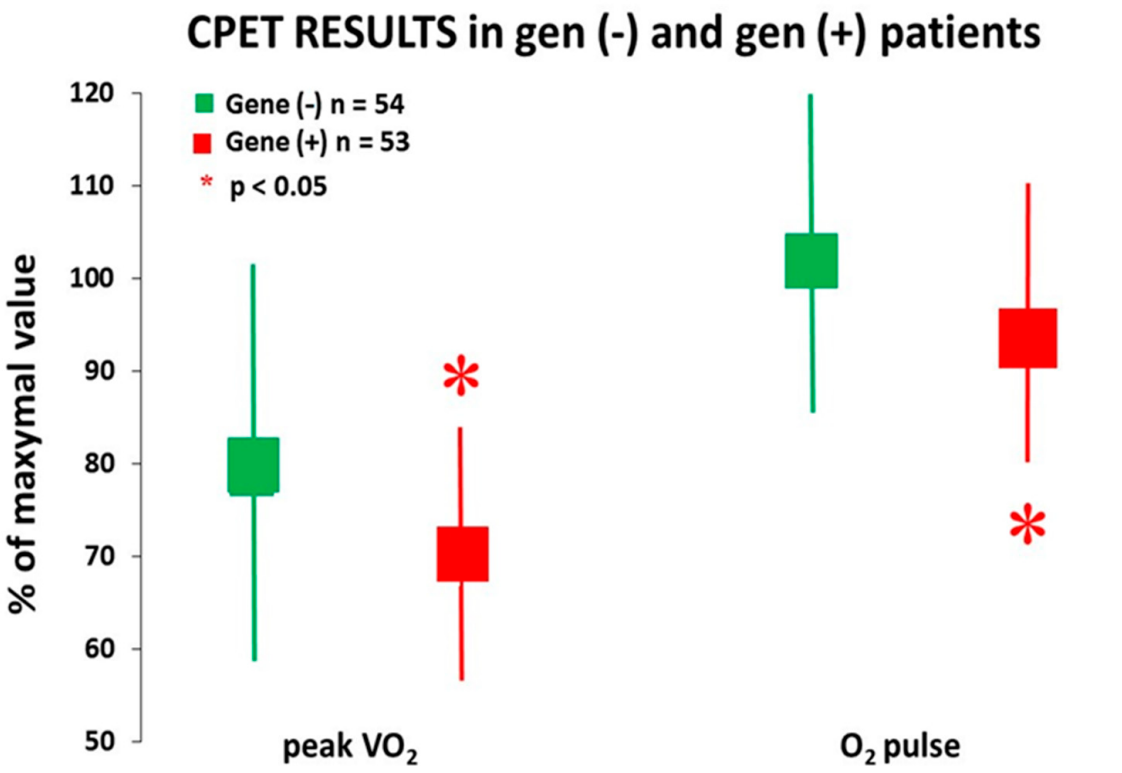


Figure 14. Peak VO2 and O2 pulse (as % of that predicted for age [116,117]) were significantly lower in gene (+) patients. See text for discussion.

Moreover, a moderate to severe reduction in performance (≤65% of the age-adjusted peak VO2) [108,109] was observed in 47% (25/53 pts) of gene (+) and only in 20% (11/54 pts) of gene (−) patients ($p < 0.05$, $\chi 2$ test).

4.2. 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 myosinbinding 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.

	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

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

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

	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.

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

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 VO2 and O2 pulse were lower, but also VO2/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 VO2) 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%) ($p < 0.05$, $\chi 2$ test).

	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

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

5. 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 [2,103]. 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 [111–113] or to the occurrence of malignant arrhythmias [110]. 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 [2,103]. 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 [2,103]. 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 [106,107]. 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 [2,103, 114-118]. 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 [105], but some disagreement is still present on the topic [119]. 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 [120]; 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 [115,117,108] and possibly with the development of severe ventricular arrhythmias [108]. 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 [109-113]. Of note, the ventilatory response to exercise was maintained (both VEVCO2 slope and P(ET)CO2 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 [109,112]. Indeed, CPET data obtained on HCM patients without heart failure are consistent with our results [110-111-113].

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 VO2). 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 VO2/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 [114-116]. 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 Ca2+ sensitivity with an early phase of hypercontractility, altered transmembrane ion transport and adverse remodelling of the sarcoplasmic reticulum [117]. 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 [118]. 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 [119], 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 [120]. Conversely, thin-filament mutations initially disrupt calcium regulation: increased Ca2+ buffering and altered handling contribute to pathogenesis via Ca2+-dependent signalling pathways [121]. So, despite genetic is not yet entered in conventionally used score for risk stratification, our study together with others already published [114-116] 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.

6. Conclusions

Cardiopulmonary test results in asymptomatic or slightly symptomatic patients with HCM show a reduced O2 consumption and O2 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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