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GENOTYPE-PHENOTYPE CORRELATIONS AND GENETIC FAMILY SCREENING IN HYPERTROPHIC CARDIOMYOPATHY

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ABBREVIATIONS

CMR = cardiac magnetic resonance

DHPLC = denaturing high performance liquid chromatography

EF = ejection fraction

DCM = dilated cardiomyopathy

HCM = hypertrophic cardiomyopathy

HF = heart failure

ICD = Implantable Cardioverter Defibrillator

LGE = late gadolinium enhancement

LV = left ventricular

LVH = left ventricular hypertrophy

LVOT = left ventricular outflow-tract

NYHA= New York Heart Association

RV = right ventricular

TDI = tissue Doppler imaging

VF = ventricular fibrillation

NSVT = non sustained ventricular tachycardia

SUMMARY

Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiac disease. Clinical presentation is heterogeneous, outcome ranging from benign asymptomatic forms to more malignant expressions resulting in sudden or heart failure death. To date, more than 450 mutations have been reported in genes encoding sarcomeric proteins, proteins of the Z-disc, intercalated discs and in genes involved in cardiac metabolism. DNA testing is helpful for confirming diagnosis in ambiguous situations, can give some prognostic information and represents the gold standard for preclinical diagnosis in family members. However, mutation screening in HCM allows positive results in a small percentage of probands and is expensive and time-consuming.

Aim of this study was to identify pathogenic mutations in the most common HCM related genes and to correlate molecular defect with clinical-morphological phenotypic pattern in a large cohort of HCM probands from a single centre. Since the mutation in the index case has been identified, a cascade screening has been performed in the first degree family members searching for carriers.

Genetic screening for the 4 most commonly HCM-related sarcomeric genes (i.e. *MYBPC3*, *MYH7*, *TNNT2*, *TNNI3*) was performed by denaturing high performance liquid chromatography (DHPLC) and direct sequencing in 83 HCM index-cases from large and/or "malignant" families. A subgroup of 30 probands underwent more extensive mutation screening by 12 genes (i.e. *MYH7, MYBPC3, MYL2, MYL3, TNNT2, TNNI3, TNNC1, TPM1, ACTC, CSRP3, PLN* and *PRKAG2*) array-based DNA resequencing assay. If the mutations found were novel, these were searched in a healthy control population to rule out the possibility that they represent single nucleotide polymorphisms (SNPs).

Including both screening methods, our population consisted of 99 index cases (age at diagnosis 31±17 years, age at last control 45±17 years, 70% males, 65% familial cases, 38% with obstruction). Twenty-seven pathogenic HCM-causing mutations were found in 30 probands (30%). Percentage of mutation positive patients was not different in probands with HCM family history (21/64 probands, 33%) versus sporadic cases (9/35 probands, 26%; p=0.46), and irrespective of the screening method used (25/83 probands, 30%, by DHPLC and direct sequencing vs 8/30 probands, 27%, by DNA resequencing array; p=0.72). In 14 index-cases screened both by DHPLC and DNA resequencing array, there was agreement between the two different screening methods

(3 mutations in 3 patients found by both methods, and just an intronic mutation "missed" by DNA resequencing array). Genes more frequently involved were *MYH7* coding for beta-myosin heavy chain (11 mutations in 11 patients) and *MYBPC3* for cardiac myosin-binding protein C (8 mutations in 12 patients). In a minority of probands HCM-causing genes were *TNNI3, TNNT2* and *MYL3*, respectively in 4, 3 and one patients. Two patients had double mutation in compound heterozygous.

Wide heterogeneity in clinical presentation and evolution was present in spite of genotype characterization, but when multiple mutations were detected, they were associated with particularly severe phenotype.

Fifty-one members from 16 different families were screened for the mutation(s) found in their family proband and 23 (45%) resulted carriers. Eight carriers had phenotypic expression fulfilling diagnostic criteria for HCM (i.e. maximal left ventricular wall thickness, MLVWT $\geq$ 13 mm), whereas 10 had only minor signs suggestive of HCM (such as ECG abnormalities, MLVWT=12-13 mm, abnormal left ventricular filling pattern at echo-Doppler), and 5 were healthy carriers. Tissue Doppler Imaging seemed to be useful for preclinical diagnosis, but a multiparametric evaluation is needed to identify mutation carriers before phenotypic expression of HCM.

Nowadays, mutation screening is becoming part of diagnostic and clinical management of HCM patients and family members. The spectrum of HCM-associated genes has moved outside the myofilaments of the sarcomere to encompass additional subgroups of proteins involved in the pathogenesis of HCM.

Mutation carriers without HCM phenotype represent a new subgroup of patients at risk for developing disease, whose clinical and prognostic profile remains unresolved, but of particular interest as possible target for preventive therapeutic strategies that can change the natural history of this disease.

RIASSUNTO

La cardiomiopatia ipertrofica (CMI) rappresenta la più frequente malattia cardiaca geneticamente determinata. È caratterizzata da un decorso clinico estremamente eterogeneo, che può variare da forme benigne ed asintomatiche a quadri particolarmente severi culminanti con morte improvvisa o per insufficienza cardiaca.

Fino ad oggi sono state identificate più di 450 diverse mutazioni a carico di oltre 20 geni codificanti non solo proteine del sarcomero, ma anche altre strutture cellulari quali il disco Z e i dischi intercari o geni implicati nel metabolismo cardiaco.

L'analisi genetica è un importante strumento diagnostico nelle situazioni dubbie, può dare talora indicazioni prognostiche, ma soprattutto consente di porre una diagnosi preclinica nei familiari di probandi affetti da CMI. Tuttavia essa consente di ottenere dei risultati conclusivi sono in una limitata percentuale di soggetti e rappresenta una metodica costosa, laboriosa ed ancora prerogativa di pochi centri specializzati.

Lo scopo di questo studio è stata l'identificazione di mutazioni patogene nei geni sarcomerici più frequentemente implicati nella CMI e la ricerca di correlazioni genotipo-fenotipo in un'ampia popolazione di pazienti con CMI seguiti presso l'ambulatorio specialistico della Clinica Cardiologica dell'Università di Padova. Una volta individuata la mutazione patogena nel probando, questa è stata ricercata nei familiari di primo grado al fine di consentire una diagnosi precoce e di programmare un adeguato follow-up clinico.

Data l'impossibilità di sottoporre sistematicamente l'intera popolazione seguita presso il nostro ambulatorio specialistico a tale indagine, si è deciso di procedere con un approccio razionale "a cascata" selezionando 83 casi-indice con forme fenotipicamente più severe o appartenenti a grandi famiglie in cui si erano verificati numerosi eventi maggiori e sottoponendo questi ad analisi genetica per screening di mutazioni nei 4 geni sarcomerici noti dalla letteratura essere i più frequentemente implicati nella CMI (*MYBPC3, MYH7, TNNT2, TNNI3)* mediante *denaturing high performance liquid chromatography* (DHPLC) e sequenziamento diretto. Un sottogruppo di 30 probandi è stato sottoposto ad analisi per ricerca di mutazioni in 12 geni sarcomerici e non (*MYH7, MYBPC3, MYL2, MYL3, TNNT2, TNNI3, TNNC1, TPM1, ACTC, CSRP3, PLN* e *PRKAG2)* mediante tecnica di *DNA resequencing array*. Qualora la mutazione trovata non fosse già nota in letteratura, questa è stata testata in una popolazione di controllo di soggetti sani, per confermare che non si trattasse di un polimorfismo.

Comprendendo entrambi i metodi di screening la nostra popolazione è risultata composta da 99 casi-indice (età media alla diagnosi 31±17 anni, età all'ultimo controllo 45±17 anni, 70% maschi, 65% con familiarità per CMI, 38% forme ostruttive). Sono state identificate 27 mutazioni patogene in 30 probandi (30%). La percentuale di probandi con mutazione è risultata non variare a seconda del metodo di screening utilizzato (25/83 probandi, 30%, identificati mediante DHPLC e sequenziamento diretto contro 8/30 probandi, 27%, mediante *DNA resequencing array*, p=0,72), né sulla base della storia familiare di CMI (21/64, 33%, nelle forme familiari, contro 9/35, 26%, nei casi sporadici, p=0,46). Dei 14 probandi indagati con entrambe le tecniche, in 3 casi le stesse mutazioni sono state identificate con entrambi i metodi, mostrando una buona concordanza diagnostica. Una sola mutazione in una regione intronica è stata identifica al DHPLC, ma "mancata" al *DNA resequencing array*. I geni più frequentemente implicati sono risultati *MYH7* codificante la catena pesante della beta-miosina con 11 mutazioni in 11 probandi e *MYBPC3* codificante la proteina C legante la miosina con 8 mutazioni in 12 probandi. Meno frequentemente sono state riscontrate mutazioni nei geni per le troponine cardiache I e T (rispettivamente in 4 e 3 pazienti) ed in un caso è stata riscontrata una mutazione nel gene *MYL3* codificante la catena leggera essenziale della miosina. In due pazienti erano presenti doppie mutazioni.

Pazienti con mutazioni a carico dello stesso gene presentavano quadri clinici e decorso estremamente variabile, particolarmente severo nei pazienti con mutazioni multiple.

In 51 familiari, provenienti da 16 famiglie, è stata ricercata la mutazione patogena trovata nel probando, e di questi 23 (45%) sono risultati portatori. Mentre in 8 pazienti alla presenza di mutazione corrispondeva espressione clinica di malattia, in 5 non vi erano segni di CMI e nei restanti 10 solamente alterazioni minori non ancora diagnostiche (alterazioni aspecifiche dell'ECG, spessore parietale del ventricolo sinistro tra 12 e 13 mm, alterato rilasciamento all'eco-Doppler). L'analisi con Doppler Tissutale si è dimostrata sensibile nell'identificazione di anomalie precoci nei portatori di mutazioni e, se inserita in un approccio diagnostico multiparametrico, potrebbe consentire una diagnosi preclinica.

In conclusione, sebbene rappresenti un'indagine costosa e che consente l'identificazione di mutazioni patogene solo in una percentuale di pazienti variabile (circa 30%) l'analisi genetica è entrata a far parte del percorso clinico-diagnostico della cardiomiopatia ipertrofica. Il numero di geni candidati e di mutazioni è in continuo sviluppo comprendendo anche numerosi geni non-sarcomerici.

Ampia variabilità clinica e fenotipica è presente nei pazienti con singola mutazione, mentre i pazienti con doppie mutazioni vanno incontro ad un decorso particolarmente severo.

Lo screening genetico nei familiari per la ricerca della mutazione identificata nel probando rappresenta il gold-standard per la diagnosi precoce e può guidare il follow-up clinico (stretta sorveglianza clinica per i portatori, rassicurazione e controlli dilazionati nei negativi). Il sottogruppo di portatori sani, il cui decorso clinico rimane ancora da chiarire, rappresenta inoltre un'interessante popolazione per studiare fenomeni precoci di comparsa della malattia ed eventuali strategie preventive.

BACKGROUND

HYPERTROPHIC CARDIOMYOPATHY

History-Definition

Although the classic appearance of asymmetrical hypertrophy of the interventricular septum was first described in 1869 by Liouville (1) and Hallopeau (2), hypertrophic cardiomyopathy (HCM) emerged as an accepted clinical entity only in the 1950s with the description of functional obstruction of the left ventricle by Sir Russell Brock (3), and of asymmetrical septal hypertrophy by Donald Teare (4). After these landmark papers, there was a period of intense clinical investigation in which the characteristic morphological and haemodynamic features of the disease were defined.

Many of the pseudonyms used for the disease during this period (such as idiopathic hypertrophic subaortic stenosis) highlight how the disorder was generally thought to be exclusively characterised by a dynamic subaortic pressure gradient (5-7). This view was reinforced by the use of M-mode echocardiography because this technique could detect septal hypertrophy and the hallmark of subaortic obstruction—systolic anterior motion of the mitral valve (8). Two-dimensional echocardiography changed this perception by showing that patterns of hypertrophy were much more heterogeneous, and that outflow obstruction at rest was present in only a minority of patients (9).

Currently, the clinical diagnosis of HCM is established with two-dimensional echocardiography by demonstrating left ventricular hypertrophy (LVH) (typically asymmetric in distribution, and showing virtually any diffuse or segmental pattern of left ventricular [LV] wall thickening) (10,11). Left ventricular wall thickening is associated with a nondilated and hyperdynamic chamber (often with systolic cavity obliteration) in the absence of another cardiac or systemic disease (e.g., hypertension or aortic stenosis) capable of producing the magnitude of hypertrophy evident, and independent of whether or not LV outflow obstruction is present (10). Although the usual clinical diagnostic criteria for HCM is a maximal LV wall thickness (MLVWT) greater than or equal to 15 mm, recent studies on genotype-phenotype correlations have shown that virtually any wall thickness (including those less than 15 mm and those within normal range) are compatible with the presence of a HCM mutant gene (11-15),

and in members of affected families also a MLVWT ≥ 13mm should be considered diagnostic for HCM (16).

Epidemiology

Although the several studies that have examined the prevalence of HCM have used very different screening methods and diagnostic criteria (17–21) almost all recorded a prevalence of about one in 500 adults (0,2%). Therefore, HCM is not rare, representing the first cause of sudden death in athletes in USA, but is relatively uncommon in routine cardiologic practice, affecting no more than 1% of outpatients (18).

The frequency of unexplained left ventricular hypertrophy in children remains unknown, but investigators have reported an annual incidence of LVH (related to all causes) between 0.3 and 0.5 per 100 000 with frequency highest in the first year of life, and greater in boys (21,22).

Morphology

Left ventricular hypertrophy

Although morphological heterogeneity in HCM is considerable, with no single pattern regarded as typical, most patients have an asymmetric pattern of hypertrophy that affects the interventricular septum more frequently than the posterolateral segments of the left ventricle (Figure 1), and in almost one third wall thickening is localized to a single segment (most often the anterior basal septum) (9,10).

A few patients show a symmetric (concentric) pattern and the apical form appear most commonly in Japanese people (23-25).

Right ventricular hypertrophy can coexist with LV disease in about 30% of HCM patients (11).

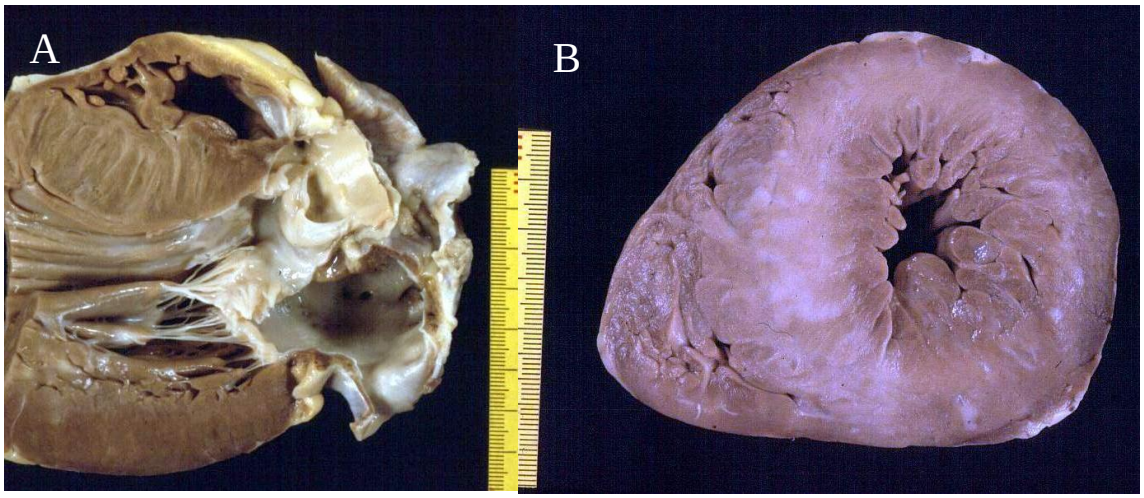

Figure 1. Gross features of HCM with asymmetrical septal hypertrophy. (A) Parasternal long axis cut of the heart with subaortic septal bulging and endocardial fibrous plaque. (B) Cross section: note the septal hypertrophy with white fibrous scar. By courtesy of Prof. Cristina Basso, Cardiovascular Pathology, University of Padua.

Left ventricular dynamic subaortic tract obstruction

Another marker of HCM not obligatory for diagnosis is a LV dynamic subaortic outflow tract or mid-cavity obstruction (LVOT) with high velocity ejection in which a variable proportion of the forward blood flow may be ejected early in systole (26). This mechanical impedance to outflow is typically produced by mitral valve leaflets systolic anterior motion (SAM) and septal contact (caused by drag effect or the Venturi phenomenon) that is responsible not only for subaortic obstruction, but also for the concomitant mitral regurgitation (usually mild-to-moderate in degree) due to incomplete leaflet apposition, which is typically directed posteriorly into the left atrium (27,28). When the mitral regurgitation jet is directed centrally or anteriorly into the left atrium, or if multiple jets are present, independent abnormalities intrinsic to the mitral valve should be suspected (e.g., myxomatous degeneration, mitral leaflet fibrosis, or anomalous papillary muscle insertion) (29,30). Occasionally (perhaps in 5% of cases), gradients and impeded outflow are caused predominately by muscular apposition in the mid-cavity region involving anomalous direct insertion of anterolateral papillary muscle into the anterior mitral leaflet, or excessive mid-ventricular or papillary muscle hypertrophy and malalignment (11,29).

Histology

Hypertrophic cardiomyopathy is characterized by disorganization of myocardial architecture with myocyte disarray, in which individual cardiomyocytes vary in size and

shape and form abnormal intercellular connections (31), usually with expansion of the interstitial compartment and areas of replacement fibrosis (Figure 2) (32,33). Cellular disarray may be widely distributed, occupying substantial portion of LV wall (always more than 5%, as diagnostic criteria).

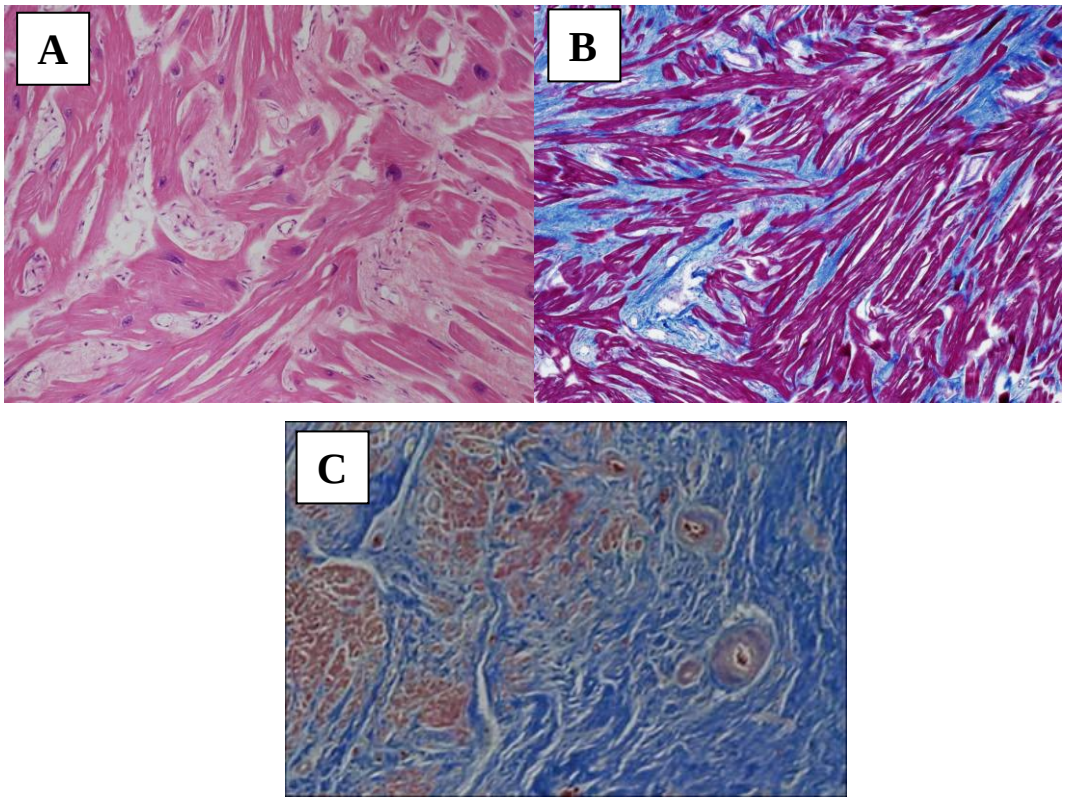

Figure 2. Microscopic features of HCM. (A) Myocyte hypertrophy and disarray with interstitial fibrosis (Haematoxylin-Eosin stain). (B) Fascicular myocyte disarray with interstitial fibrosis (Heidenhain's Trichrome stain). (C) Interstitial and replacement fibrosis with intramural coronary arteries showing markedly thickened walls and narrowed lumen. By courtesy of Prof. Cristina Basso, Cardiovascular Pathology, University of Padua.

Another common feature is small vessel disease, in which intramural coronary vessels are apparently narrowed by medial hypertrophy (34-36). The clinical significance of these microvascular changes is uncertain, but along with features such as increased myocardial mass and reduced capillary density, they almost certainly contribute to the development of myocardial ischemia leading to myocyte death and repair in the form of patchy or transmural replacement scarring in some patients (37,38).

Presence of myocardial bridges can also be responsible for intramural coronary artery compression during systole that cause or worsen ischemic events (39-42).

Disorganized cellular architecture, myocardial scarring and expanded interstitial collagen matrix may serve as arrhythmogenic substrates predisposing to electrical instability, source of life-threatening events (ventricular tachycardia and/or fibrillation), that represent the predominant mechanisms of sudden death (43).

Pathophysiology

The most important pathophysiological mechanisms (closely linked one to each other) responsible for clinical presentation and disease progression in HCM are three:

1. Diastolic dysfunction
2. Myocardial ischemia
3. Presence of dynamic LV outflow tract gradient

Diastolic dysfunction

Despite this heterogeneity, almost all patients with HCM have some degree of diastolic dysfunction and the presence of subtle changes in LV filling may even identify patients with preclinical disease without LV hypertrophy and in some patients, diastolic abnormalities can dominate the clinical picture to resemble restrictive cardiomyopathy (44-46).

The origin of diastolic dysfunction in HCM is both multifactorial and complex, with changes at the molecular, myocardial tissue, and global LV levels. Morphological factors that influence the degree of diastolic dysfunction include the degree of ventricular hypertrophy, myocardial disarray, and interstitial fibrosis (47). These may be modulated by expression of growth factors and cytokines. Ventricular shape and geometry, including the presence of small LV systolic volumes and LV cavity obliteration, also may lead to reduced LV distensibility. Their effect on impaired diastolic filling is partially offset by the presence of increased elastic recoil. These changes may explain why dynamic diastolic pressure-volume curves measured during filling in patients with HCM often are considerably shallower than would be anticipated if one assumed high chamber stiffness (48).

Diastolic dysfunction has an important clinical role, causing increase of LV filling pressure that leads to pulmonary oedema and dyspnea. It is also responsible for left atrial enlargement predisposing to atrial fibrillation (AF) (49).

Myocardial ischemia

The presence of ischemia in HCM is well documented both from anatomical and histological point of view (40) and clinically manifested as angina during exertion or *post-prandium* (caused by large meals).

Left ventricular hypertrophy, with or without outflow tract obstruction, and structural myocardial alterations, such as interstitial fibrosis, disarray and microvascular disease, myocardial bridges, as well as the mismatch between myocardial mass and coronary circulation are likely responsible for impaired coronary vasodilator reserve and bursts of myocardial ischemia (37-40,50). In addition to the above mechanisms, impaired LV relaxation and increased LV end-diastolic pressure can compress the coronary microcirculation and further restrict coronary artery blood flow (51-54). Myocardial ischemia can be induced by exercise, vasodilators such as adenosine, and dobutamine.

Acute-subacute ischemic events, as well as chronic myocardial ischemia, are recognized as playing an important role in the course of the disease: acute ischemic events may act as a trigger for electrical instability and consequent malignant arrhythmias, whereas chronic or recurrent ischemia may lead to the replacement fibrosis responsible for disease progression (51), severe heart failure and death (34,55)

Presence of dynamic LV outflow tract gradient

Left ventricular outflow tract gradient characteristics and mechanisms have been previously described.

Obstruction in HCM is characteristically dynamic (i.e., not fixed): the magnitude (or even presence) of an outflow gradient may be spontaneously labile and vary considerably with a number of physiologic alterations (as a heavy meal or ingestion of a small amount of alcohol) (56,57). Different gradient cut-offs have been proposed for segregating individual patients into hemodynamic subgroups, but rigorous partitioning into such hemodynamic categories according to gradient can be difficult because of the unpredictable dynamic changes that may occur in individual patients (56). Nevertheless, it is reasonable to divide the overall HCM disease spectrum into hemodynamic subgroups, based on the representative peak instantaneous gradient as assessed with continuous wave Doppler:

1) *obstructive gradient under basal (resting) conditions* equal to or greater than 30 mm Hg (2.7 m/s by Doppler),

2) *latent (provocable) obstructive* - gradient less than 30 mm Hg under basal conditions and equal to or greater than 30 mm Hg with provocation,

3) *nonobstructive* - less than 30 mm Hg under both basal and provocable conditions.

Outflow gradients are responsible for a loud apical systolic ejection murmur associated with a constellation of unique clinical signs (27).

Although it has previously been subject to periodic controversy (26), there is now widespread recognition that the subaortic gradient (30 mm Hg or more) and associated elevations in intra-cavity LV pressure reflect true mechanical impedance to outflow and are of pathophysiologic and prognostic importance to patients with HCM (58). Indeed, LVOT obstruction is a strong, independent predictor of disease progression to HCM related death, to severe symptoms of New York Heart Association (NYHA) class III or IV, and to death due specifically to heart failure and stroke (58).

Disease consequences related to chronic outflow gradients are likely to be mediated by the resultant increase in LV wall stress, myocardial ischemia and eventually cell death and replacement fibrosis (58-60). Therefore, the presence of LVOT obstruction justifies intervention to reduce or abolish significant subaortic gradients in severely symptomatic patients who are refractory to maximum medical management (61).

Diagnosis

Clinical examination

Hypertrophic cardiomyopathy may be initially suspected on auscultation because of a systolic murmur at the left sternal edge, radiating to the aortic and mitral areas, but not to the neck or axilla, that characteristically increases with actions that reduce preload or afterload, such as standing, Valsalva manoeuvre, or amyl nitrate inhalation. This auscultatory finding is limited to patients with dynamic outflow tract obstruction and often is associated with presence of a pansystolic murmur radiating to the axilla suggesting mitral regurgitation.

In patients with dynamic left ventricular outflow tract obstruction, the arterial pulse has a rapid upstroke and a rapid downstroke, sometimes followed by a palpable reflected wave resulting in a bisferiens pulse character. Examination of jugular venous pulsation can reveal a prominent a wave caused by reduced right ventricular compliance. The left ventricular impulse can be sustained with a palpable atrial beat. More rarely, a triple impulse with an additional late systolic impulse can be felt (62).

Electrocardiography

The 12-lead ECG is abnormal in 75% to 95% of HCM patients and typically demonstrates a wide variety of patterns (15,63) .

The most frequent changes include left atrial enlargement, repolarisation abnormalities, and pathological Q-waves, most commonly in the inferolateral leads. Voltage criteria for left ventricular hypertrophy alone are non-specific and are often seen in normal young adults (63). Giant negative T-waves in the mid-precordial leads are characteristic of the so-called Japanese variant of the disease, in which hypertrophy is mainly confined to the apical segments of left ventricle (25). Some patients have a short PR interval with a slurred QRS upstroke that is not usually (except of non-sarcomeric *PRKAG2* and *LAMP2* mutation related disease) associated with Wolff-Parkinson-White syndrome (62).

The commonest arrhythmias recorded during ambulatory monitoring are premature ventricular complexes, non-sustained ventricular tachycardia (NSVT) and supraventricular tachyarrhythmias such as AF and flutter (64-68).

Clinical presentation with sustained ventricular tachycardia (VT) is rare, except possibly in some patients with apical left ventricular aneurysms (69)

Despite low specificity, ECG abnormalities in HCM have diagnostic value in raising a suspicion of HCM in family members without LVH on echocardiogram and in athletes undergoing preparticipation screening.

Echocardiography

Clinical diagnosis of HCM is established most easily and reliably with two-dimensional echocardiography by demonstrating left ventricular hypertrophy but nondilated LV chamber (often with systolic cavity obliteration) in the absence of another cardiac or systemic disease (e.g., hypertension or aortic stenosis) capable of producing the magnitude of hypertrophy evident, and independent of whether or not LV outflow obstruction is present (11).

All echocardiographic imaging windows should be used to accurately define the areas of increased wall thickness. Hypertrophied segments often have slightly increase brightness in comparison with segments having normal end-diastolic wall thickness. LV hypertrophy, although usually asymmetric, can also be concentric. The distribution of hypertrophy can be in any pattern and at any location, including the right ventricle. Although septal predominance is more common, hypertrophy can be isolated to the LV

free wall or apex. When the extent of hypertrophy is difficult to visualize, having a high index of suspicion and meticulous imaging of the LV apex and/or the use of LV cavity opacification by intravenous contrast aids in the accurate diagnosis (70).

Transthoracic echocardiography (TTE) combined with the intravenous injection of an echocardiographic contrast agent should be performed in patients with HCM with suspected apical hypertrophy, to define the extent of hypertrophy and to detect apical aneurysms and clots (70-72).

It is possible to express the severity of hypertrophy using semiquantitative scores (73) which are based on wall thickness measurements by two-dimensional (2D) imaging in parasternal short-axis views at end-diastole (i.e. relative wall thickness = septal wall thickness + posterior wall thickness/ left ventricular end diastolic diameter), but in the presence of adequate-quality images and expertise, three-dimensional (3D) echocardiography provides the most accurate echocardiographic approach for quantifying LV mass.

Echocardiography evaluation in patients with HCM can also provide assessment of LV volumes and ejection fraction (EF). Traditionally, the biplane Simpson's method has been applied to the measurement of LV volumes (74), but recently, real-time 3D echocardiography has been shown to provide more accurate means of quantification (75) though there is a paucity of data on its accuracy in HCM. LV EF is usually normal or increased in patients with HCM. Overt LV systolic dysfunction, termed the "dilated or progressive phase of HCM", "end-stage HCM", or "burnt-out HCM", is usually defined as an LV EF < 50% and occurs in a minority (2%–5%) of patients. Prognosis is markedly worse in the presence of LV systolic dysfunction (76). Likewise, the development of an apical aneurysm is an uncommon but important complication that can be readily recognized with contrast echocardiography (72).

In addition to 2D and 3D imaging, Doppler methods have been used to assess for the presence of subclinical LV systolic dysfunction. Tissue Doppler Imaging (TDI) measures the velocity of myocardial motion in systole and in diastole. Reduced systolic (Sa) and reduced early diastolic (Ea or E') velocities can occur before the onset of overt hypertrophy (77-79). Doppler tissue imaging can also be used to measure myocardial strain and strain rate, which unlike TDI velocities are not affected by translation and tethering. Strain rate imaging has been shown to be useful in differentiating nonobstructive HCM from hypertensive LV hypertrophy (80). However, tissue Doppler–derived strain imaging has technical limitations due to its angle dependence.

Speckle-tracking echocardiography directly assesses myocardial motion from B-mode (2D) images and is independent of angulation between the ultrasound beam and the plane of motion. Several studies have shown reductions in strain in patients with HCM compared with controls (81,82).

The assessment of LV diastolic function in HCM can be limited by the relatively weak correlations between the mitral inflow and pulmonary venous flow velocities and invasive parameters of LV diastolic function (83). Previous studies have noted reasonable correlations between E/Ea ratio and LV filling pressures (84). The E/Ea ratio has also been correlated with exercise tolerance in adults (85) and children (86) with HCM. In addition, septal Ea velocity appears to be an independent predictor of death and ventricular arrhythmia in children with HCM (86).

A comprehensive approach is recommended when predicting LV filling pressures in patients with HCM, taking into consideration the above velocities and ratios, as well as pulmonary artery pressures and LA volume, particularly in the absence of significant mitral regurgitation and AF, as the latter two conditions lead to LA enlargement in the presence of a normal LA pressure (70).

About 25% of patients have dynamic left ventricular outflow tract obstruction caused by contact between the anterior, or less commonly the posterior, mitral valve leaflet (or chordal apparatus) and the interventricular septum during systole (62). The maximal instantaneous gradient, reflecting the severity of LVOT obstruction, is determined by measuring the peak LVOT velocity by continuous-wave Doppler. Care should be taken to avoid contamination of the LVOT signal with the mitral regurgitation jet. Distinguishing a dynamic LVOT gradient from fixed LVOT obstruction by a subvalvular membrane is important and concomitant aortic valve stenosis should be excluded by examination of the aortic valve anatomy.

Midcavitary obstruction can occur with and without LVOT obstruction in ventricles with hyperdynamic function and/or concentric hypertrophy. This is frequently observed in elderly patients with a sigmoid septum. The site of obstruction is determined by pulsed wave and color Doppler showing high velocities at the site of obstruction (velocity aliasing by pulsed-wave Doppler) (70).

Occasionally, anomalous papillary muscles that insert directly into the mitral leaflet can also cause obstruction of the LVOT (87).

Most patients with dynamic outflow tract obstruction have a posteriorly directed jet of mitral regurgitation caused by failure of leaflet apposition which can be significant

(moderate or greater depending on the extent of the gap). Mitral regurgitation is dynamic in HCM and is affected by the same factors that influence the severity of obstruction, but not all mitral regurgitation associated with HCM is related to SAM. Patients with HCM, in fact, can have intrinsic valvular abnormalities, such as mitral valve prolapse, leaflet thickening secondary to injury from repetitive septal contact or turbulent regurgitation jet, chordal rupture, chordal elongation or thickening, and infectious etiologies (70).

Echocardiography can also be used for guidance of septal reduction procedures (i.e. septal myectomy or alcohol septal ablation).

Nuclear imaging

Currently, the use of nuclear imaging for the sole purpose of assessment of cardiac structure in HCM patients is no more recommended since echocardiography and CMR have higher spatial resolution and provide accurate measurements.

Otherwise, nuclear imaging can play a role in detection of myocardial ischemia so that, while routine performance of stress perfusion imaging in conjunction with single photon-emission computed tomography (SPECT) is not recommended, in HCM patients with chest pain and low probability of CAD, stress SPECT imaging can be considered.

In patients with HCM with normal coronary arteries, myocardial perfusion positron emission tomography (PET) studies have shown that although resting myocardial blood flow, may be similar to normal control subjects, the augmentation of blood flow with vasodilation (e.g., dipyridamole) may be significantly blunted. In addition, such abnormal myocardial blood flow reserve with vasodilatation was shown to be more pronounced in the subendocardial regions (88,89). Such quantifiable abnormalities in myocardial blood flow reserve, in the absence of epicardial CAD, could be attributed to myocardial ischemia from microvascular dysfunction and have prognostic importance (51). Patients with HCM with the greatest attenuated myocardial blood flow responses to dipyridamole were more likely to subsequently develop LV remodeling, decreased LV EF, and severe heart failure symptoms (70).

Cardiovascular Magnetic Resonance

In the last decade, Cardiovascular Magnetic Resonance (CMR) has emerged as an important 3D tomographic imaging technique, which provides images of the heart at high spatial and temporal resolution, in any plane and without ionizing radiation (90).

Allowing at the same time morphological, dynamic, perfusional and tissue characterization images of the heart, CMR represents a “one shop stop” technique.

Current cine CMR imaging sequences are breath-hold and retrospectively or prospectively electrocardiographically gated acquisitions acquired in nearly identical imaging planes as that of 2D echocardiography. Furthermore, LV short-axis stacks are thin myocardial slices (typically 7 mm) providing complete tomographic coverage of the entire myocardium.

Cine imaging sequences produce sharp contrast between the bright blood pool and the dark myocardium and therefore can provide detailed characterization of the HCM phenotype, including accurate wall thickness measurements (91,92) and highly reproducible measurements of ventricular volumes and mass.

CMR is particularly useful for characterizing the presence, location, and extent of LV hypertrophy in HCM. Although MLVWT measurements are often similar between echocardiography and CMR, focal regions of increased wall thickness may not be well visualized by 2D echocardiography but can be detected by CMR in a subset of patients with localized HCM (93). The basal anterolateral free wall is one location in the left ventricle where hypertrophy may not be well seen by echocardiography, because the lateral epicardial border in this region is difficult to differentiate from the adjacent thoracic parenchyma in the short-axis orientation (92). The LV apex is another region of the myocardium where CMR may provide an advantage over echocardiography in identifying hypertrophy (93). Likewise, CMR can identify the presence of apical aneurysms in patients with HCM, which can have management implications (94). Therefore, CMR imaging should be considered in the evaluation of patients with HCM in whom the LV myocardium is not well visualized.

CMR in HCM can be useful in RV evaluation, since, with this technique, it has been demonstrated that up to one third of patients have increased RV wall thickness and mass, (95) and if the septomarginal area is involved, RV outflow tract obstruction may be observed.

Furthermore, CMR assessment of papillary muscles has provided insight into the mechanism of outflow obstruction by demonstrating that the presence of an apically displaced anterolateral or double bifid papillary muscle is associated with a significantly higher likelihood of having a resting LVOT gradient (96).

CMR measurements of ventricular volumes and EF are accurate and reproducible. In addition, quantitative measurements of regional systolic and diastolic function can be obtained using myocardial tagging methods (70).

CMR can also characterize the magnitude of LVOT dynamic obstruction by application of phase velocity flow-mapping sequences to determine peak velocity, but at the present time, CMR-derived velocities can be assessed only under basal conditions.

Moreover, by CMR it is possible to visualize the mitral valve apparatus and assess the severity and mechanism of mitral regurgitation.

An important application of CMR is perfusion study, with accurate qualitative and quantitative assessment of myocardial blood flow at rest and during pharmacologic stress (typically using adenosine). Stress CMR has demonstrated blunted myocardial blood flow in response to vasodilator stress in patients with HCM, which appears greater in the subendocardial layer in comparison with the subepicardial layer and is present in both hypertrophied and nonhypertrophied segments (70).

Among all, probably the most innovative and promising application of CMR is myocardial tissue characterization. Contrast-enhanced CMR with late gadolinium enhancement (LGE) sequences can detect areas of focally abnormal myocardial substrate in patients with HCM (60, 97-100). Areas of LGE can be planimetered and the amount quantified and expressed as a percentage of total LV mass. Some reports in native hearts after transplantation of patients with end stage HCM have demonstrated concordance between in vivo LGE CMR images and gross and histopathologic evidence of fibrosis (99). The prevalence of LGE in HCM is approximately 50% to 80% and when present occupies on average 10% of the overall LV myocardial volume (60, 97-100). There is no specific pattern of LGE characteristic for HCM, although predominately the distribution of LGE in HCM does not correspond to a coronary vascular distribution, as in patients who have had myocardial infarctions. LGE is most often located in the ventricular septum but not uncommonly can be confined to only the LV free wall or insertion points of the RV free wall and ventricular septum and is more common in segments with hypertrophy and in patients with HCM with larger LV mass indices.

A number of studies have demonstrated a relationship between the extent of LGE and adverse LV remodeling associated with systolic dysfunction. The magnitude of LGE is greatest in patients with HCM in the end-stage phase of the disease (EF < 50%) (55, 60, 97-100). However, it is still unclear if the extent of LGE can be used to prospectively

identify patients with HCM at risk for progressing toward systolic dysfunction. Likewise, a number of cross-sectional studies have demonstrated a significant association between the presence of LGE and ventricular tachyarrhythmias on ambulatory 24-hour Holter electrocardiography (101,101), but is not clear whether the extent of LGE provides greater predictive value in identifying patients with HCM at risk for sudden death compared with only the presence of LGE. Prognostic data with regard to LGE and cardiovascular outcome have now been evaluated in recent prospective short-term studies (55,98,100). A significant relationship was observed between LGE and either sudden death or appropriate implantable cardioverter defibrillator (ICD) discharge (100) in a more recent study. However, given the small number of adverse events, it is necessary to obtain longer follow-up in larger study cohorts to have the statistical robustness necessary to determine if LGE is indeed an independent predictor of adverse events in HCM.

Moreover contrast-enhanced CMR can accurately quantify the amount of tissue necrosis after septal ablation as well as provide important information regarding the relationship between the location of scarring and LVOT morphology, and although the routine performance of CMR after septal reduction therapy is not recommended, it can be of value in selected patients when questions arise about LV function and remodeling after the procedure that could not be satisfactorily answered by echocardiography, or when gradients recur late after the procedure (70).

Screening and preclinical diagnosis

In HCM family members periodic screening is recommended at intervals of every 12 months during adolescence and every 5 years in adults (103) as well as at the onset of symptoms suggestive of HCM.

Whereas ECG represents the most diffuse, easily available, and inexpensive first-step screening tool for HCM, at the present time, echocardiography is the most practical and accurate technique for HCM diagnosis. All myocardial segments, not only the septum, should be carefully examined for evidence of hypertrophy on these screening examinations and also mild LV hypertrophy in any segment (i.e. MLVWT $\geq$ 13 mm) should be take into consideration.

Studies in transgenic animal models have noted the presence of abnormal myocardial function before the development of hypertrophy (104). These observations have led to the investigation of Doppler tissue imaging in the preclinical diagnosis of HCM in

individuals carrying sarcomeric protein mutations causing HCM and some studies have shown annular Ea velocity to be promising (77-79) whereas limitations to this approach include the lower specificity in older individuals or those with coexisting disease. Therefore, abnormal Doppler velocities do not establish the diagnosis of HCM but can help identify gene carriers who may benefit from closer follow-up.

Cardiovascular magnetic resonance should be considered in patients with technically challenging echocardiograms, and in patients in whom electrocardiographic results is or have become abnormal, with still normal results on echocardiography.

A variety of potential morphologic abnormalities identified by CMR may be present in preclinical patients with HCM. Specifically, crypts localized predominately in the inferior septum have been demonstrated by CMR in preclinical patients, although the aetiology of these structural abnormalities remains uncertain (105).

Cascade genetic analysis remains the gold standard for early identification of mutation carriers in family with HCM. Find a pathogenic mutation in the index case has the potential of providing the diagnostic gold standard for his/her offspring, siblings, parents and more distant relatives. A positive genetic test would then enable systematic scrutiny of the index case's relatives to separate the 'haves' from the 'have nots' (positive vs. negative test). In other words, the genetic testing of the index case stratifies the family enabling 2 very different courses to be charted: 1) close surveillance of the genotype-positive, pre-clinical individual; and 2) casual observation or dismissal of the genotype-negative/phenotype-negative relative and his/her future progeny.

In general, and irrespective of genetic testing, once a diagnosis of HCM has been performed, all first-degree relatives and probably 'athletic' second-degree relatives to the index case should be screened by an electrocardiogram and echocardiogram (106).

Different screening approaches, based either on genetic, or to traditional clinical methods, have been compared and molecular strategy, despite initial higher cost has proved to be more effective, identifying more individuals at risk (107).

At present, however, there are several obstacles to the translation of genetic research into practical clinical applications and routine clinical strategy. These include the substantial genetic heterogeneity, the low frequency with which each causal mutation occurs in the general HCM population, and the important methodological difficulties associated with identifying a single disease-causing mutation among several different genes in view of the complex, time-consuming, and expensive laboratory techniques involved. The current development of better methodologies for automated, direct DNA

sequencing now provides sensitive techniques that can accurately define the molecular cause for HCM in a single proband, without involving family members or complex linkage analysis in large pedigrees (11).

Clinical course

Hypertrophic cardiomyopathy is unique among cardiovascular disease with the potential for clinical presentation during any phase of life from infancy to old age.

The clinical course is typically variable, and patients may remain stable over long periods of time with up to 25% of a HCM cohort achieving normal longevity (75 years of age or older) (11).

However, the course of many patients may be punctuated by adverse clinical events, largely related to sudden, unexpected death, embolic stroke, and the consequences of heart failure.

Hypertrophic cardiomyopathy is also a rare cause of severe heart failure in infants and very young children, and presentation in this age group itself constitutes an unfavourable prognostic sign.

In general, adverse clinical course proceeds along one or more of several of the following pathways, which ultimately dictate treatment strategies:

1) high risk for premature sudden and unexpected death;
2) progressive symptoms largely of exertional dyspnea, chest pain (either typical of angina or atypical in nature), and impaired consciousness, including syncope, near-syncope or presyncope (i.e., dizziness/lightheadedness), in the presence of preserved LV systolic function;
3) progression to advanced congestive heart failure (the “end-stage phase”) with LV remodeling and systolic dysfunction;
4) complications attributable to AF, including embolic stroke.

Recently, Melacini et al (108) have described three clinicopathological profiles of heart failure in HCM, predominantly associated with end-stage systolic dysfunction (EF<50%), LVOT obstruction at rest and non-obstructive forms with preserved systolic function and prevalent diastolic dysfunction. Atrial fibrillation contributed to heart failure in more than 60% of patients among the three profiles.

However selection biases have had an important impact on our perceptions of this disease in fact, much of the published clinical data assembled over four decades have

emanated largely from a few selected tertiary centers in North America and Europe, disproportionately comprised of patients referred because of their high-risk status or severe symptoms requiring highly specialized care (such as surgery). Over-dependence on frequently cited, ominous mortality rates of 3% to 6% per year for HCM-related premature death from tertiary centers may have led to an exaggeration of the overall risk and impact of this disease on patients (11). More recent reports from non-tertiary centers are probably more representative of the overall disease state, citing annual mortality rates in a much lower range of about 1%. Nevertheless, there are subgroups of patients within the broad HCM spectrum with annual mortality rates of up to 6% per year for whom a correct risk stratification is needed (108).

Exertional dyspnea and disability (often associated chest pain), dizziness, presyncope and syncope usually occur in the presence of preserved systolic function and a nondilated LV. Symptoms appear to be caused in large measure by diastolic dysfunction with impaired filling due to abnormal relaxation and increased chamber stiffness, leading in turn to elevated left atrial and LV end-diastolic pressures (with reduced stroke volume and cardiac output), pulmonary congestion, and impaired exercise performance with reduced oxygen consumption at peak exercise (109).

The pathophysiology of such symptoms, due to this form of diastolic heart failure, may also be intertwined with other important pathophysiologic mechanisms such as myocardial ischemia, with or without outflow obstruction associated with mitral regurgitation, and AF. Patients with LV outflow obstruction are more disabled by elevated LV pressures and concomitant mitral regurgitation than by diastolic dysfunction, as is evidenced by the often dramatic symptomatic benefit derived from major therapeutic interventions that reduce or obliterate outflow gradient (most frequently myectomy or alcohol ablation) (11).

Chest pain in the absence of atherosclerotic coronary artery disease (CAD) may be typical of angina pectoris or atypical in character. Most chest discomfort is probably due by bursts of myocardial ischemia, whose mechanisms have been previously described. Because typical anginal chest pain may be part of the HCM symptom-complex, associated atherosclerotic CAD (which may complicate clinical course) is often overlooked in these patients. Therefore, coronary arteriography is indicated in patients with HCM and persistent angina who are over 40 years of age or who have risk factors for CAD, or when CAD is judged possible prior to any invasive treatment for HCM such as septal myectomy (or alcohol septal ablation) (11).

Sudden death risk stratification

Sudden and unexpected death (SD) has been recognized as the most devastating and often unpredictable complication and the most frequent mode of premature demise from this disorder. The high-risk HCM patients constitute only a minority of the overall disease population, and historically, this subset of patients have represented a major investigative focus.

Sudden cardiac death may occur as the initial disease presentation, most frequently in asymptomatic or mildly symptomatic young people, it often occurs without reliable warning signs or symptoms, and often in the early morning hours after awakening (11). Although SD is most frequent in adolescents and young adults less than 30 to 35 years old, such risk also extends through mid-life and beyond (110); the basis for this particular predilection of SD for the young is unresolved. Sudden cardiac death occurs most commonly during mild exertion, but it is not infrequently triggered by vigorous physical exertion (111). Indeed, HCM is the most common cause of cardiovascular related SD in young people, including competitive athletes (112).

The available data (largely from recorded arrhythmic events that triggered appropriate defibrillator interventions) suggest that complex ventricular tachyarrhythmias emanating from an electrically unstable myocardial substrate are the most common mechanism by which SD occurs in HCM (11). Ventricular arrhythmias on routine ambulatory (Holter) 24-h ECG monitoring are an important clinical feature in adults with HCM. Alternatively, it is possible that in some patients supraventricular tachyarrhythmias could trigger ventricular tachyarrhythmias or that bradyarrhythmias occur and require back-up pacing.

It has been suggested that life-threatening tachyarrhythmias could be provoked in HCM by environmental factors (e.g., intense physical exertion) or be, alternatively, intrinsic to the disease process, involving a vicious cycle of increasing myocardial ischemia and diastolic (or systolic) dysfunction, possibly worsened by outflow obstruction, systemic arterial hypotension, or supraventricular tachyarrhythmias which lead to decreased stroke volume and coronary perfusion.

Although the available data on the stratification of SD risk are substantial and a large measure of understanding has been achieved, it is important to underscore that precise identification of all individual high-risk patients by clinical risk markers is not completely resolved. Nevertheless, it is possible to identify most high-risk patients by non-invasive clinical markers (113-115), and only a small minority (about 3%) of those

HCM patients who die suddenly are without any of the currently acknowledged risk markers.

The highest risk for SD has been associated with the following (11):

1) prior cardiac arrest or spontaneously occurring and sustained VT;
2) family history of a premature HCM-related SD particularly if sudden, in a close relative, or if multiple in occurrence;
3) unexplained syncope, particularly in young patients or when exertional or recurrent;
4) nonsustained VT (of 3 beats or more and of at least 120 beats/min) evident on ambulatory (Holter) ECG recordings;
5) abnormal blood pressure response during upright exercise which is attenuated or hypotensive, indicative of hemodynamic instability, and of greater predictive value in patients less than 50 years old or if hypotensive;
6) extreme LVH with MLVWT of 30 mm or more, particularly in adolescents and young adults
7) identification of a high-risk mutant gene .

Hypertrophic cardiomyopathy patients should undergo comprehensive clinical assessments on an annual basis for risk stratification and evolution of symptoms, including careful personal and family history, noninvasive testing with two-dimensional echocardiography (primarily for assessment of magnitude of LVH and outflow obstruction), 24- or 48-h ambulatory (Holter) ECG recording for ventricular tachycardia, and blood pressure response during maximal upright exercise (treadmill or bicycle).

Subsequent risk analysis should be performed periodically and when there is a perceived change in clinical status.

The concept that risk of SD is related to the magnitude of hypertrophy does not, however, infer that the risk is necessarily low when MLVWT is less than 30 mm, because other risk markers may be present in a given patient; indeed, the majority of patients who die suddenly do, in fact, have MLVWT of less than 30 mm. Furthermore, a small number of high-risk pedigrees with troponin T and I mutations have been reported in whom SD was associated with particularly mild forms of LVH, including a few individuals with normal LV wall thickness and mass (44,116,117).

Although prognosis is generally not tightly linked to the pattern and distribution of LVH, the preponderance of evidence suggests that segmental wall thickening at the low

end of the morphologic spectrum (i.e., less than 20 mm thickness, regardless of its precise location), generally confers a favorable prognosis in the absence of other major risk factors (61,118). Such localized hypertrophy includes the nonobstructive form of HCM confined to the most distal portion of LV ("apical HCM") (11).

Disorganized cardiac muscle cell arrangement, myocardial replacement scarring as a repair process following cell death and the expanded interstitial (matrix) collagen compartment probably serve as the arrhythmogenic substrate predisposing some susceptible patients to re-entrant, life-threatening ventricular tachyarrhythmias.

It is a clinical perception that the premonitory symptom most associated with the likelihood of SD in HCM is syncope or near-syncope (119). However, the sensitivity and specificity of syncope as a predictor of SD is low, possibly because there are many potential causes of syncope, some of which are unrelated to the basic disease state and are often neurocardiogenic in origin. Even when an underlying cause for impaired consciousness cannot be identified, this symptom complex can be compelling in some HCM patients, particularly when it is exertional or recurrent.

Available data suggest that LV outflow obstruction can only be regarded as a minor risk factor for SD in HCM (58). The impact of gradient on SD risk is not sufficiently strong (positive predictive value of only 7%) for obstruction to merit a role as the sole (or predominant) deciding clinical parameter and the primary basis for decisions to intervene prophylactically with an ICD.

One report suggests that short-tunneled (bridged) intramyocardial segments of left anterior descending coronary artery independently convey increased risk for cardiac arrest, probably mediated by myocardial ischemia (120). However, potential biases in patient selection, the frequency of coronary arterial bridging in surviving adults and those who have died of noncardiac causes, and the need for routine invasive coronary arteriography in order to identify this abnormality prospectively seem to mitigate the potential power of coronary bridging as a risk factor for SD (42).

It has been proposed, based on genotype-phenotype correlations, that the genetic defects responsible for HCM could represent important determinants and stratifying marker of prognosis both for SD and heart failure risk, with specific mutations conveying either favorable or adverse prognosis (i.e., high- and low-risk mutations). For example, it has been suggested that some cardiac beta-myosin heavy chain mutations (such as Arg403Gln and Arg719Gln) and some troponin-T mutations are associated with higher incidence of premature death (116), decreased life expectancy, and early onset disease

manifestations, while other HCM genes such as cardiac myosin-binding protein C or alpha-tropomyosin convey a more favourable prognosis (13).

However, routine clinical testing for specific mutations believed to be high (or low) risk has been shown to have low yield (121). Therefore, it is premature to draw definitive conclusions regarding gene-specific clinical outcomes based solely on the presence of a particular mutation, by virtue of extrapolation from available epidemiologic-genetic data which are formulated from relatively small numbers of genotyped families largely skewed toward high-risk status (11).

The particular prognosis attached to adult carriers with a mutant HCM gene but without LVH and clinical expression of HCM is uncertain; however, at the present time, this subgroup would not appear to be associated with an adverse prognosis (122).

There is no convincing evidence that invasive markers such as those defined with laboratory electrophysiologic testing (i.e., programmed ventricular stimulation) have an important routine role in identifying those HCM patients who have an unstable electrical substrate and are at high-risk for SD due to life-threatening arrhythmias. Electrophysiologic studies with or without programmed ventricular stimulation may, however, have some value in selected patients such as those with otherwise unexplained syncope (11).

Most of the clinical markers of SD risk in HCM are limited by relatively low positive predictive values due in part to relatively low event rates. However, the high negative predictive values (at least 90%) of these markers suggest that the absence of risk factors and certain other clinical features can be used to develop a profile of patients having a low likelihood of SD or other adverse events (11).

Patients with an apparently favorable prognosis in the absence of risk factors constitute an important proportion of the overall HCM population. Most such patients probably will not require aggressive major medical treatment and generally deserve a large measure of reassurance regarding their prognoses. Little or no restriction is necessary with regard to recreational activities and employment, although exclusion from intense competitive sports is advised (123).

Prevention of sudden death and ICD

Historically, treatment strategies to prophylactically reduce the risk for SD have been predicated on the administration of drugs such as beta-adrenergic blockers, verapamil, and type I-A antiarrhythmic agents (i.e., quinidine, procainamide and amiodarone) to

those patients perceived to be at high risk. However, there is no evidence that this practice of prophylactically administering such drugs empirically to asymptomatic HCM patients is efficacious in mitigating the risk for SD; furthermore, because of its potential toxicity, amiodarone is unlikely to be tolerated throughout long periods and this strategy now seems out-dated with the current availability of measures that more effectively prevent SD, such as the ICD.

When risk level for SD is judged by contemporary criteria to be unacceptably high and deserving of intervention, the ICD is the most effective and reliable treatment option available, harboring the potential for absolute protection (124-126).

In one multicenter retrospective study, ICDs appropriately sensed and automatically aborted potentially lethal ventricular tachyarrhythmias by restoring sinus rhythm in almost 25% of a high-risk cohort, followed for a relatively brief period of 3 years (124). Appropriate device interventions occurred at a rate of 11% per year for secondary prevention and at 5% per year for primary prevention, usually in patients with no or only mild prior symptoms. Patients receiving appropriate defibrillation shocks were generally young (mean age 40 years). Implantable cardioverter defibrillators often remained dormant for prolonged periods before discharging (up to 9 years), emphasizing the unpredictable timing of SD events in this disease, the potentially long risk period, and the requirement for extended follow-up duration to assess survival in HCM studies (125).

The ICD is strongly warranted for secondary prevention of SD in those patients with prior cardiac arrest or sustained and spontaneously occurring VT (124).

Although multiple risk factors convey increasingly greater risk for SD, a single major risk factor in a individual patient may be sufficient to justify strong consideration for primary prevention with an ICD (11). Therefore such management decisions must often be based on individual judgment for the particular patient, by taking into account the overall clinical profile including age, the strength of the risk factor identified, the level of risk acceptable to the patient and family, and the potential complications largely related to the lead systems and to inappropriate device discharges.

There is, at present, an understandable reluctance on the part of pediatric cardiologists to implant such devices chronically in children (particularly for primary prevention) considering the necessary, ongoing commitment required for maintenance and the likelihood that lead or other (ICD-related) complications will occur over very long time periods. However, while adolescence may represent a psychologically difficult age to be

encumbered by an ICD, it should also be emphasized that this is coincidently the period of life consistently showing the greatest predilection for SD in HCM. One alternative but empiric strategy proposed for some very young high-risk children is the administration of amiodarone as a bridge to later ICD placement after sufficient growth and maturation has occurred.

Some investigators also regard the end-stage phase of HCM as a risk factor for SD, justifying implantation of an ICD during the waiting period prior to the availability of a heart for transplant.

Accordingly with the recommendations of International Societies of Sport Cardiology (123,127), young patients with HCM should be restricted from intense competitive sports to reduce the risk for SD that may be associated with such extreme lifestyle.

However, stringent lifestyle or employment modifications for other HCM patients (who are not participants in organized athletics) do not seem justified or practical, although intense physical activity involving burst exertion (e.g., sprinting) or systematic isometric exercise (e.g., heavy lifting) should be discouraged.

Although data are scarce, there is presently no evidence to suggest that genetically affected but phenotypically normal family members are generally at increased risk for SD. Therefore, there is little basis for subjecting such individuals to the same activity restrictions as many other HCM patients, or excluding them from competitive athletics in the absence of cardiac symptoms, family history of SD, or a mutant gene regarded as malignant. However, periodic (probably annual) noninvasive clinical evaluation directed toward risk assessment is warranted in this subset of patients.

Medical treatment

Obstructive form

Beta-blockers are the first line treatment in patients with LVOT obstruction. Propranolol was the first drug used in the medical management of HCM, and agents such as atenolol, metoprolol, or nadolol have been employed more recently. The majority of patients show improvement upon treatment although high doses are often required. Side effects, however, can limit utility as well as induce pharmacological chronotropic incompetence, blunting the heart rate response to exercise and causing symptomatic deterioration.

Non-dihydropiridinic calcium channel blockers like verapamil is best avoided in individuals with obstruction because of possible peripheral vasodilatation and haemodynamic collapse (128).

The negative inotropic and type I-A antiarrhythmic agent disopyramide has been evaluated more systematically and is also effective in gradient and symptom reduction, probably because of negative inotropism. It may have a superior effect on exercise tolerance compared to beta-blockers. They are, however, best used in combination as disopyramide alone tends to accelerate AV node conduction and increase the potential risk from supraventricular arrhythmias. Disopyramide should be administered in the maximum tolerated dose (300 to 600 mg per day); the limiting factor is usually the anticholinergic side effects (128). Although disopyramide incorporates antiarrhythmic properties, there is little evidence that proarrhythmic effects have intervened in HCM patients. Nevertheless, this issue remains of some concern in a disease associated with an arrhythmogenic LV substrate; prolongation of the QT interval should be monitored while administering the drug.

Furthermore, disopyramide administration may be deleterious in nonobstructive HCM by decreasing cardiac output, causing most investigators to limit its use to patients with outflow obstruction who have not responded to beta-blockers or verapamil (11).

Non-obstructive form

Agents such as beta-blockers or non-dihydropiridinic calcium channel blockers like verapamil and diltiazem are used to treat chest pain and dyspnoea and improve exercise tolerance.

The beneficial effects of beta-blockers on symptoms of exertional dyspnea and exercise intolerance appear to be attributable largely to a decrease in the heart rate with a consequent prolongation of diastole and relaxation and an increase in passive ventricular filling. These agents lessen LV contractility and myocardial oxygen demand and possibly reduce microvascular myocardial ischemia (11).

Verapamil in doses up to 480 mg per day (usually in a sustained release preparation) has favorable effects on symptoms, probably by virtue of improving ventricular relaxation and filling as well as relieving myocardial ischemia and decreasing LV contractility (129,130).

The response can be suboptimal although those patients with severe chest pain often benefit from high doses of verapamil or diltiazem.

Pulmonary congestion has been treated with diuretics but there is a risk of decompensation in individuals with severe diastolic dysfunction. Diuretics should only be used judiciously and if possible only in the short term, as chronic prescription tends to result in a reduction in stroke volume and cardiac output that ultimately lowers exercise capacity (128).

Heart failure (end-stage phase)

A small but important subgroup of patients with nonobstructive HCM develops systolic ventricular dysfunction and severe heart failure, usually associated with LV remodeling demonstrable as wall thinning and chamber enlargement. The subset should receive treatment for conventional cardiac failure, including angiotensin converting enzyme (ACE) inhibitors, beta-blockers, digoxin, spironolactone, and if necessary cardiac transplant.

Atrial fibrillation

Atrial fibrillation is the most common sustained arrhythmia in HCM (66-68). Paroxysmal episodes or chronic AF ultimately occur in 20% to 25% of HCM patients, linked to left atrial enlargement and an increasing incidence with age. Clinical cohort studies show that AF is reasonably well tolerated by about one-third of patients and is not an independent determinant of sudden unexpected death (67); however, it is possible that in certain susceptible patients, AF may trigger life-threatening ventricular arrhythmias. Nevertheless, AF is independently associated with heart failure-related death, occurrence of fatal and nonfatal stroke, as well as long-term disease progression with heart failure symptoms (66-68).

Risk for complications of AF is enhanced when the arrhythmia becomes chronic, onset is before 50 years of age, and outflow obstruction is present (67).

Paroxysmal episodes of AF may also be responsible for acute clinical deterioration, with syncope or heart failure resulting from reduced diastolic filling and cardiac output, as a consequence of increased ventricular rate and with loss of atrial contraction (and its contribution to ventricular filling) in a hypertrophied LV with pre-existing impaired relaxation and compliance. Atrial fibrillation in HCM should be managed generally in accordance with the ACC/AHA guidelines (131). In particular, electrical or pharmacologic cardioversion are indicated in those patients presenting within 48 h of onset, assuming that the presence of atrial thrombi can be excluded with a reasonable

degree of certainty. Amiodarone is generally regarded as the most effective antiarrhythmic agent for preventing recurrences of AF (132).

A generally aggressive strategy for maintaining sinus rhythm is warranted in HCM because of the association of AF with progressive heart failure and mortality, as well as stroke. In chronic AF, beta-blockers, verapamil (and digoxin) have proved effective in controlling heart rate, although A-V node ablation and permanent ventricular pacing may occasionally be necessary in selected patients.

Anticoagulant therapy (with warfarin) is indicated in patients with either paroxysmal or chronic AF (11,67,68,131).

Because even one or two episodes of paroxysmal AF have been associated with increased risk for systemic thromboembolization in HCM, the threshold for initiation of anticoagulant therapy should be low and can include patients after the initial AF paroxysm. Such clinical decisions should be tailored to the individual patient after considering the risk for hemorrhagic complications, lifestyle modifications, and expectations for compliance.

At present, there is little experience specifically in HCM patients with emerging and novel alternative treatment strategies for AF such as pulmonary vein radio-frequency ablation, the surgical MAZE procedure, or implantable atrial defibrillators to warrant definitive recommendations at this time (11).

Infective endocarditis prophylaxis

In HCM there is a small risk for bacterial endocarditis, which appears largely confined to those patients with LV outflow tract obstruction under resting conditions or with intrinsic mitral valve disease (133). Therefore, the AHA recommendation (134) should be applied to HCM patients with evidence of outflow obstruction under resting or exercise conditions at the time of dental or selected surgical procedures that create a risk for blood-borne bacteremia.

Non-medical treatment

In some patients, medical therapy ultimately proves insufficient to control symptoms, and the quality of life becomes unacceptable to the patient. At this point in the clinical course, after administration of maximum drug treatment, the subsequent therapeutic strategies are dictated largely by whether LV outflow obstruction is present

Surgery

Patients in a small subgroup (about 5% of all HCM patients), are regarded as candidates for surgery. These patients have particularly marked outflow gradients (peak instantaneous usually greater than or equal to 50 mm Hg), as measured with continuous wave Doppler echocardiography either under resting/basal conditions and/or with provocation. In addition, these patients have severe limiting symptoms, usually of exertional dyspnea and chest pain that are regarded in adults as NYHA functional classes III and IV, refractory to maximum medical therapy (11).

Over the past 40 years, the ventricular septal myectomy operation (also known as the Morrow procedure) has become established as a proven approach for amelioration of outflow obstruction and the standard therapeutic option, and the gold standard, for both adults and children with obstructive HCM and severe drug-refractory symptoms (135-142). The myectomy operation should be confined to centers experienced in this procedure.

Myectomy is performed through an aortotomy and involves the resection of a carefully defined relatively small amount of muscle from the proximal septum (about 5 to 10 g), extending from near the base of the aortic valve to beyond the distal margins of mitral leaflets (about 3 to 4 cm), thereby enlarging the LV outflow tract and, as a consequence in the vast majority of patients, abolishing any significant mechanical impedance to ejection and mitral valve SAM immediately normalizing LV systolic pressures, abolishing mitral regurgitation, and ultimately, reducing LV end-diastolic pressures. Such an abrupt relief of the gradient with surgery (in contrast to slower reduction with alcohol septal ablation in many cases) is particularly advantageous in patients with severe functional limitations.

Some surgeons have utilized a more extensive myectomy procedure, with the septal resection widened and extended far more distally than in the classic Morrow procedure (143). In addition, the anterolateral papillary muscle may be dissected partially free from its attachment with the lateral LV free wall to enhance papillary muscle mobility and reduce anterior tethering of the mitral apparatus (144). Alternatively, mitral valve replacement or repair has been employed in selected patients judged to have severe mitral regurgitation (30).

Currently, surgical centers experienced with myectomy do not advocate mitral valve replacement, but mitral valvuloplasty (plication) in combination with myectomy has

been proposed for some patients with particularly deformed or elongated mitral leaflets (145).

Published reports of over 2000 patients from North American and European centers show remarkably consistent results with the ventricular septal myectomy operation: isolated myectomy is now performed with low operative mortality in patients of all ages, including children, at those centers having the most experience with this procedure (1% to 3%, and even less in the most recent cases) (11, 140-142,146). Surgical risk may be higher among very elderly patients (particularly those with severe disabling symptoms associated with pulmonary hypertension), and patients undergoing additional cardiac surgical procedures.

Complications such as complete heart block (requiring permanent pacemaker) and iatrogenic ventricular septal perforation have become uncommon (equal to or less than 1% to 2%), while partial or complete left bundle-branch block is an inevitable consequence of the muscular resection and is not associated with adverse sequelae (135-146).

Septal myectomy is associated with persistent, longlasting improvement in disabling symptoms and exercise and decreased frequency of syncope five or more years after surgery, while the effect of surgery per se on longevity is unresolved, although several surgical series have reported improved late survival after myectomy (138,142,146).

Percutaneous alcohol septal ablation

An alternative to surgery is the more recently developed alcohol septal ablation technique (147-150). First reported in 1995, this catheter interventional treatment involves the introduction of absolute alcohol (about 1 to 3 cc of desiccated ethanol at 95% concentration) into a target septal perforator branch of the left anterior descending coronary artery for the purpose of producing a myocardial infarction (equivalent to an area of necrosis estimated to be 3% to 10% of the LV mass) within the proximal ventricular septum.

Septal ablation mimics the hemodynamic consequences of myectomy by reducing the basal septal thickness and excursion (producing akinetic or hypokinetic septal motion), enlarging the LV outflow tract and, thereby, lessening the SAM of the mitral valve and mitral regurgitation. Whereas alcohol septal ablation may trigger a rapid reduction in resting outflow gradient evident in the catheterization laboratory, more frequently, a progressive decrease in the gradient occurs after 6 to 12 months, usually achieving levels in a range equivalent to that with myectomy, and resulting from remodeling of

the septum without significant impairment in global LV ejection (147-150). The mortality and morbidity associated with alcohol ablation in experienced centers have proved to be relatively low, although they are similar in surgical myectomy.

Reports of permanent pacemaker implantation for induced high-grade A-V block have ranged from 5% to as high as 30% (11), but this complication appears to be decreasing substantially with the use of smaller amounts of alcohol. In contrast to septal myectomy, which usually produces left bundle branch block, alcohol ablation commonly results in right bundle branch block. It is also possible for coronary artery dissection to occur, as well as backward extravasation of alcohol, producing occlusion or abrupt coronary no-flow (151) and a large anteroseptal myocardial infarction. A predominate concern raised with respect to alcohol septal ablation is the potential long-term risk for arrhythmia-related cardiac events (including SD) directly attributable to the procedure. Unlike myectomy, alcohol septal ablation potentially creates a permanent arrhythmogenic substrate in the form of a healed intramyocardial septal scar that could increase the risk of lethal re-entrant arrhythmias.

While alcohol ablation represents a selective alternative to surgery, it is not at this time regarded as the standard and primary therapeutic strategy for all severely symptomatic patients refractory to maximal medical management with marked obstruction to LV outflow and septal myectomy remains the gold standard for this HCM patient subset (59, 61,152,153).

Dual-chamber pacing.

Despite some investigations reported dual-chamber pacing to be associated with a substantial decrease in outflow gradient (154,155), as well as amelioration of symptoms in most patients, two subsequent randomized, cross-over, double-blind studies reported the effects of pacing in HCM patients to be less favourable than the observational data had suggested (156,157), and currently guidelines have designated pacing for severely symptomatic and medically refractory HCM patients with LV outflow obstruction as a class IIB indication (11).

GENETICS

Sarcomeric and non-sarcomeric genes mutations

Since the 1980s, the growing recognition that most HCM cases were familial led to a determined effort to identify the underlying genetic defect. The initial genetic studies in 1989 established a linkage with the chromosome 14q (158), identifying two attractive albeit unexpected candidate genes, *MYH6* and *MYH7*, that encode the α and β myosin heavy chains (α and β MyHC), respectively. Both genes were robustly expressed in the heart and separated by only 3600 bp on chromosome 14, (159) and the complete nucleotide sequence of *MYH7* was already published. Gene sequence analysis followed the linkage studies and revealed a missense variant in exon 13 of *MYH7* that substituted a highly conserved arginine residue (403) with glutamine (denoted Arg403Glu) in a large English family and a missense variant (Arg453Cys) in another HCM family (160). The identification of rare nonsynonymous variants that segregated with HCM in unrelated families (160-162), which altered residues that were highly conserved during evolution and were absent from hundreds of control samples, provided compelling evidence that *MYH7* mutations caused HCM. But analyses of *MYH7* in many other HCM families were unrevealing of other mutations, and new linkage searches began to identify additional disease genes. HCM loci were mapped genes to chromosome 1q3 (163) and 15q2 (164). The disease gene encoded at 1q3 was recognized to be troponin T (*TNNT2*) and the 15q2 region was known to encode α-tropomyosin (165), one of several thin filament sarcomere proteins that regulates actinomyosin interactions.

Since then, many mutations in these and other sarcomeric, thick or thin filament proteins with contractile, structural, or regulatory functions, have been reported (Table 1), leading to the modern notion that the HCM is a disorder of the myocyte contractile apparatus (Figure 3).

Table 1. HCM sarcomeric genes

GENE	LOCUS	PROTEIN	FREQUENCY(%)
MYH7	14q11.2–q12	Beta-myosin heavy chain	15–25
MYBPC3	11p11.2	Cardiac myosin-binding protein C	15–25
TNNT2	1q32	Cardiac troponin T	<5
TNNI3	19p13.4	Cardiac troponin I	<5
TPM1	15q22.1	Alpha-tropomyosin	<5
MYL2	12q23–q24.3	Ventricular regulatory myosin light chain	<2
MYL3	3p21.2–p21.3	Ventricular essential myosin light chain	<1
TNNC1	3p21.3–p14.3	Cardiac troponin C	<1
ACTC	15q14	Alpha-cardiac actin	<1
TTN	2q24.3	Titin	<1
MYH6	14q11.2–q12	Alpha-myosin heavy chain	<1

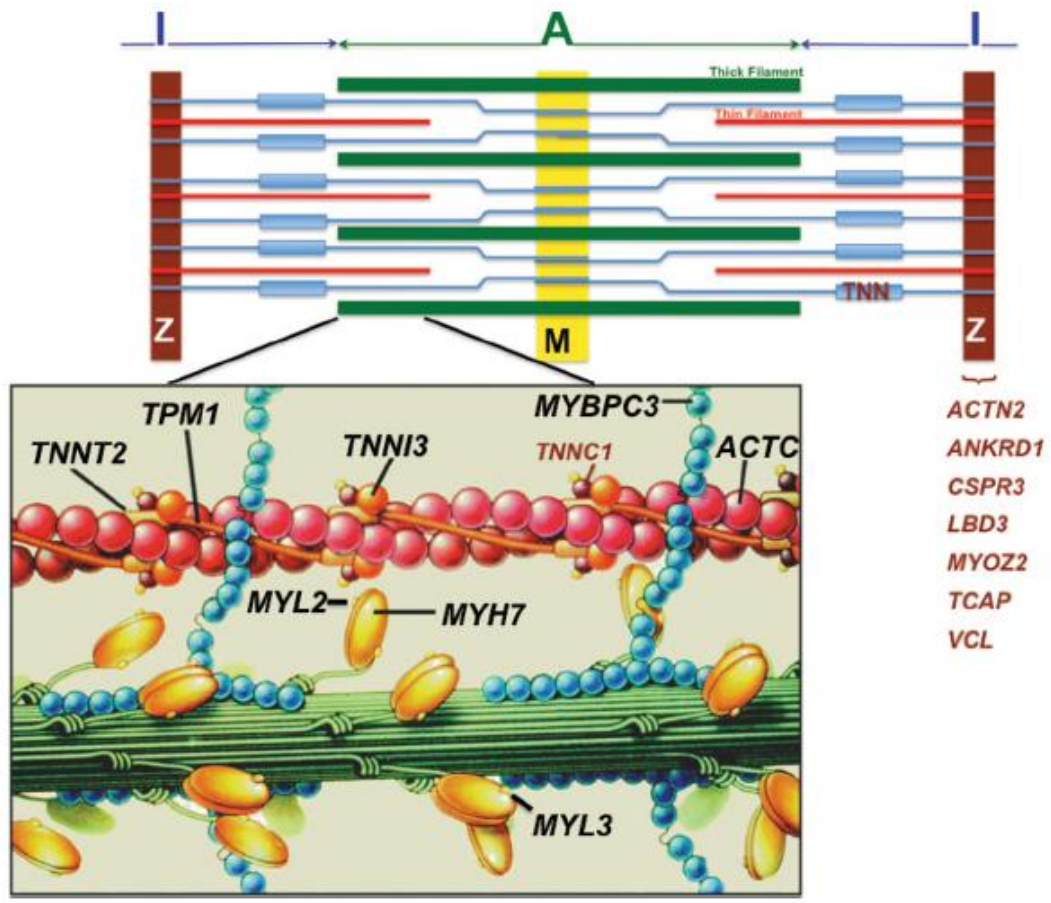

Figure 3. Schematic representation of a sarcomere (the unit of contraction that spans 2 neighboring Z-bands) is shown with the locations of thin and thick filaments in relationship to I, A, and H bands. The detailed representation of the A band highlights definitive HCM genes (black) that encode β myosin heavy chain (*MYH7*), myosin binding protein-C (*MYPBC3*),

troponin T (*TNNT2*), α tropomyosin (*TPM1*), myosin regulatory light chain (*MYL2*), myosin essential light chain (*MYL3*), and alpha-cardiac actin (*ACTC*). Additional genes that have been implicated in HCM (brown) encode Z-disc proteins, troponin C (*TNNC1*), titin (*TTN*), α myosin heavy chain (*MYH6*) (not depicted), and phospholamban (*PLN*) (not depicted). From Seidman CE and Seidman JG. *Circ Res* 2011;108:743-750 (166).

Expanding the scope of proteins involved in the pathogenesis of HCM, the spectrum of HCM-associated genes has moved outside the myofilaments of the sarcomere to encompass additional subgroups that could be classified as *'Z-disc-HCM'* and *'calcium-handling HCM'* (Table 2 and 3).

Table 2. HCM Z-disc genes.

GENE	LOCUS	PROTEIN	FREQUENCY(%)
CSRP3	11p15.1	Muscle LIM protein	<1
TCAP	17q12–q21.1	Telethonin	<1
LBD3	10q22.2–q23.3	LIM binding domain 3 (alias: ZASP)	1-5
ACTN2	1q42–q43	Alpha-actinin 2	<1
ANKRD1	10q23.31	Cardiac ankyrin repeat protein (CARP)	<1
NEXN	1p31.1	Nexilin	<1
VCL	10q22.1–q23	Vinculin/metavinculin	<1
MYOZ2	4q26–q27	Myozenin 2	<1

Table 3. HCM calcium-handling genes

GENE	LOCUS	PROTEIN	FREQUENCY(%)
JPH2	20q12	Junctophilin-2	<1
PLN	6q22.1	Phospholamban	<1

Due to its close proximity to the contractile apparatus of the myofilament, its specific structure-function relationship with regard to cytoarchitecture, as well as its role in the stretch-sensor mechanism of the sarcomere, attention subsequently focused on the cardiac Z-disc. This focus has been fuelled by the evidence that HCM and dilated cardiomyopathy (DCM) are partially allelic disorders, in which mutations in the same genes—especially the Z-disc—can be responsible for both cardiomyopathic phenotypes (167-171). The first Z-disc mutations associated with HCM were described in muscle

LIM protein encoded by *CSRP3* (168) and telethonin encoded by *TCAP* (169). Recently, *LDB3*-encoded LIM domain binding 3, *ACTN2*-encoded alpha actinin 2, *VCL*-encoded vinculin/metavinculin (170,171), and *MYOZ2*-encoded myozenin-2 (172) have been added to that list. In another demonstration of mutations in one gene causing multiple diseases, *MYPN*-encoded myopalladin (*MYPN*) mutations were implicated in the pathogenesis of DCM, HCM, and restrictive cardiomyopathy (RCM) via disturbed myofibrillogenesis, abnormal gene expression, and/or abnormality in assembly of Z-disc and intercalated disc (E. Purevjav, T. Arimura, S. Augustin, et al., unpublished data, June 2009).

In yet another signal transduction pathway, proteins involved in calcium-induced calcium release and the hypothesis that errors in this process may lead to compensatory hypertrophy have always been of high interest in the pathogenesis of HCM. Mutations have been described in the promoter and coding region of *PLN*-encoded phospholamban, an important inhibitor of cardiac muscle sarcoplasmic reticulum Ca^{2+}-ATPase (173,174). Recently it has been shown that mutations in *JPH2*-encoded junctophilin 2 (*JPH2*), which helps approximate the sarcoplasmic reticulum calcium release channels and plasmalemmal L-type calcium channels (175), as well as mutation in other Ca^{2+} regulatory genes (i.e. *CALR3*-encoded calreticulin, and *CASQ2*-encoded calsequestrin) may cause HCM (176).

Among these last genes, only that encoding myozenin-2 (172) and actinin (177) were identified from unbiased genomic analyses, and novel variants in each were demonstrated to exhibit statistically significant segregation within HCM families. Whether the variants identified in other posited HCM genes are truly pathogenic remains less certain and warrants further confirmation, especially given the recent recognition that each human genome contains approximately 1000 rare nonsynonymous variants, including premature stop signals (166).

After about 20 years of genetics in HCM, more than 1000 distinct protein gene mutations have been identified, and HCM is considered a genetic cardiac disease inherited as a mendelian autosomal dominant trait with incomplete penetrance and variable expression.

Myofilaments *MYBPC3* and *MYH7* remain so far the most common HCM-associated genes, with an estimated prevalence of 15% to 25% for both genes.

Anyway, among the HCM-associated genes the prevalence of mutations has ranged from 35% to 65% in several different international cohorts of unrelated patients who

met the clinically accepted definition of HCM (178-181), therefore more is still to learn about genetics in this disease.

HCM phenocopies (or HCM as a part of multisystem diseases)

In some cases HCM is associated with other clinical signs or symptoms like ventricular pre-excitation or muscle weakness. These diseases are sometimes referred to as "phenocopies" (apparent similar disorder with different causes), and the most important ones are listed in Table 4.

Table 4. HCM phenocopies Modified from Bos et al *J Am Coll Cardiol* 2009;54:201-11 (106).

GENE	LOCUS	PROTEIN	SYNDROME
GAA	17q25.2–q25.3	Alpha-1,4-glucosidase deficiency	Pompe's disease
GLA	Xq22	Alpha-galactosidase A	Fabry's disease
AGL	1p21	Amylo-1,6-glucosidase	Forbes disease
FXN	9q13	Frataxin	Friedrich's ataxia
PTPN11	12q24.1	Protein tyrosine phosphatase, nonreceptor type 11, SHP-2	Noonan's syndrome, LEOPARD syndrome
RAF1	3p25	V-RAF-1 murine leukemia viral oncogene homolog 1	Noonan's syndrome, LEOPARD syndrome
TAZ	Xq28	Tafazzin (G4.5)	Barth syndrome/LVNC
DTNA	18q12	Alpha-dystrobrevin	Barth syndrome/LVNC

Some diseases presenting mainly with cardiac hypertrophy turn out to have clearly distinct underlying pathophysiologies. In 2001, two independent groups discovered γ-subunit of AMP-activated protein kinase *(*AMPK encoded by *PRKAG2*), an important regulator of cellular energy homeostasis, mutations being involved in families with cardiac hypertrophy and ventricular pre-excitation, conduction abnormalities, and signs of Wolff-Parkinson-White syndrome (182-184). In 2005, Arad et al. (185) also described mutations in lysosome-associated membrane protein-2 encoded by *LAMP2* and AMPK and found that underlying metabolic diseases mimicked the clinical phenotype of HCM.

Yang et al. (186) showed that lysosome-associated membrane protein 2 (*LAMP2)* mutations may account for a significant portion of patients diagnosed with pediatric or

juvenile onset HCM, especially when skeletal myopathy and/or Wolff-Parkinson-White syndrome are present ("Danon's disease") (187).

Also mitochondrial DNA or transfer RNA mutations exhibit HCM-like feature, causing defect in cardiac energy metabolism, and these can be part of syndromic diseases (MERRF, MELAS, LHON) .

Phenocopies or rare variants pose a tough dilemma for the clinician. If the phenotype does not look like typical HCM and other symptoms are present, the presence of an underlying multisystem disease should be considered, and additional clinical testing should be performed. On the other hand, if myofilament genetic testing does not reveal an HCM-associated mutation, testing for mutations in the metabolic genes can reveal that the LVH is the primary presentation of a multisystem disease process.

Effect of gene mutations

Several models have been proposed regarding the mechanisms by which dominant mutations alter the sarcomere structure and function. These include inactivation of one allele so that the amount of functional protein is reduced (haploinsufficiency); formation of a mutant protein that interferes with normal protein function (dominant negative effect); and formation of a mutant protein that has acquired novel functions. Most HCM gene mutations produce missense codons that replace the normal amino acid with another amino acid. Less commonly, HCM mutations encode truncations or alter splice signals that either cause haploinsufficiency (13) or cause short in-frame insertions and deletions. These aberrant transcripts may encode stable mutant proteins that are incorporated into cardiac myofilaments (188). With the presence of stable mutant proteins in the sarcomere, an important question that emerges is whether HCM arises because protein function is impaired or because new functions arise.

Studies of mutations in myosin and myosin-binding protein C (MyBP-C) have been particularly informative in answering these questions. A series of early *in vitro* studies utilized heterologous expression of mutant fragments of myosin that showed a significant decrease in the actin sliding speed as measured in unregulated *in vitro* motility assay (189-191). These results supported the hypothesis that mutations in the myosin components cause a decrease in the motor function of sarcomere and a presumably compensatory hypertrophic response.

Analyses of animal models that were engineered to carry a human HCM mutations have allowed further assessment and interpretation of the consequences of these defects. Mutations in myosin heavy chain (192,193), MyBP-C (194), and troponin T (195) have been genetically engineered into genomes of mice and rabbits. These models recapitulated many characteristics of the human disease, including cardiac hypertrophy, myocyte disarray, increased fibrosis, and altered cardiac physiology. Analyses of expression of mutant proteins in these HCM models indicate that haploinsufficiency is not the mechanism for disease. Rather, HCM arises from the dominant effects of mutant proteins on sarcomere function.

Biophysical studies of HCM models have employed in vivo hemodynamics, analyses of isolated cardiac muscle fibers, and single molecule studies of purified myosin; these investigations have suggested conclusions different from those obtained with early *in vitro* studies.

Analyses of the HCM mouse model that carries the R403Q human mutation in the α MyHC (MHC403/+) (192) revealed enhanced actin-activated myosin ATPase activity, increased generated force, and accelerated actin filament sliding. These data indicate that the R403Q mutation produced a gain in myosin functions rather than a decrease in motor function (196). Similar results were found in a related study utilizing human myosin isolated from cardiac punch biopsies from patients with either the R403Q or the L908V mutations (197). Taken together, these findings suggest a molecular mechanism for the supranormal cardiac performance that is often evident in human HCM.

Further support for a "gain of function" mechanism has been provided by in vitro motility assays performed on purified human cardiac myosin isolated from a donor heart with a homozygous R403W mutation (198). These studies also showed disproportionate enhancement of mechanical and enzymatic properties of human mutant myosin and inefficient ATP utilization (198).

While the controversy over whether sarcomere protein mutations cause reduced or augmented myosin motor function has not been completely resolved, there is consensus that the initial step in the pathogenetic mechanism of HCM is incorporation of mutant protein with altered mechanical properties to the normal sarcomere. How this triggers hypertrophy and myofibrillar disarray is less clear. Presumably, the heterogeneity of mutant and wild-type myosin proteins within the sarcomere would uncouple the normal mechanical coordination between myosin heads and the enhanced ATPase activity that might result in higher levels of energy consumption (13). Increased energy consumption

combined with decrease energy supply as a result of impaired blood flow to the hypertrophied heart could cause premature myocytes death and replacement fibrosis, a well-recognized histopathologic feature HCM (199).

Some workers have suggested that abnormal myocardial bioenergetics are the final common pathophysiological pathway for all HCM gene mutations (183,200). Evidence lending support to this idea includes the association between mitochondrial DNA mutations and hypertrophy (201,202), the identification of low ATP hydrolysis sufficient to compromise sarcoplasmic reticulum Ca-ATPase activities in mice with mutations in α-myosin heavy chain, and the association between AMP kinase gene mutations and hypertrophy (183) (Figure 4).

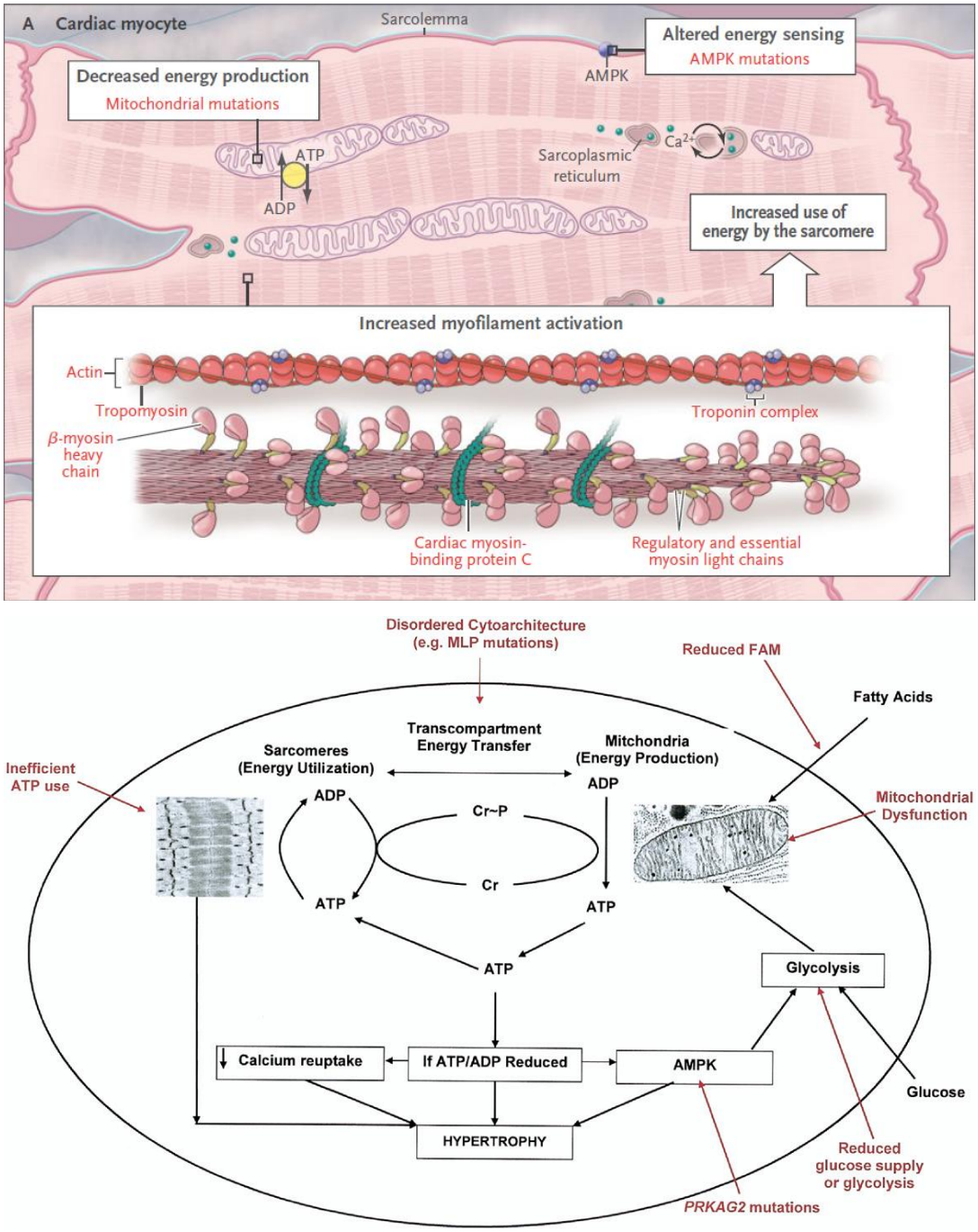

Figure 4. Pathogenesis of Hypertrophic Cardiomyopathy. In HCM, mutations in sarcomeric proteins generally increase myofilament activation and result in myocyte hypercontractility and excessive energy use. Alterations in myocardial energy status can also result from primary mutations affecting myocardial energy generation (e.g., mitochondrial mutations). These mitochondrial defects and mutations in the cardiac energy-sensing apparatus (e.g., AMP-activated protein kinase [AMPK]) recapitulate a HCM-like phenotype. Alterations in myocardial energetics and in calcium handling combined with stimulation of signaling pathways diminish myocyte relaxation and promote myocyte growth, with aberrant tissue

architecture (i.e., myofibrillar disarray and fibrosis). From Watkins et al. *N Engl J Med* 2011;364:1643-56 (203) and Ashrafian and Watkins *J Am Coll Cardiol* 2007;49:1251-64 (204).

Phosphorous-31 magnetic resonance spectroscopy has shown that, on average, the ratio of cardiac phosphocreatine to ATP (an index of cellular energy status) is 30% lower in patients positive for Troponin T, α-MyHC, and MyBP-C with hypertrophic cardiomyopathy than in controls (205). However, abnormal ATP use is also seen in diseases such as congestive cardiac failure and ischaemic heart disease, so although energy homoeostasis might contribute to the pathophysiology of hypertrophic cardiomyopathy, whether it is a primary or secondary event is unclear (62).

Genotype-phenotype correlations

Clinical diversity of HCM can reflect the broad spectrum of distinct underlying molecular cause, or genetic heterogeneity, but studies of HCM families indicate that clinical variability of disease expression exists also among individuals with identical mutations (206). Despite the first genetic studies of HCM suggested that particular gene abnormalities were associated with specific clinical phenotypes, defining precise genotype–phenotype correlations is not feasible, limited by the low frequency of each mutation and, at present, consistent data are available for only a few mutations in the most common affected genes.

However, some specific mutations have been recognized with strong clinical influence. Among affected individuals from different families, hypertrophy is generally extensive and a higher incidence of sudden cardiac death has been observed in β MyHC gene mutations. In particular, β MyHC missense mutations R403Q, R453C, and R719W are associated with severe clinical course (207). In contrast, some mutations in the MyBP-C gene are associated with delayed clinical expression, so that onset of hypertrophy occurs late in adulthood (179). Mutations in the thin filaments component, particularly the cardiac troponin T (cTnT) gene, are associated with high incidence of sudden cardiac death, primarily in young males, even with only mild hypertrophy (207,116). Cardiac actin mutations (particularly the E101K missense mutation) are associated with apical form of HCM, an uncommon variant of nonobstructive hypertrophy localized to the cardiac apex with benign clinical course (208).

With definition of the precise genetic cause for HCM, there has been increased recognition of patients who carry more than one mutation. The dose of mutant protein appears to have a strong influence on disease expression, in that individuals with homozygous or compound heterozygous mutations in sarcomere protein genes have earlier onset and often exceptionally severe phenotypes (209).

Modifier genes and environmental factors

Phenotypic expression of HCM (i.e., LVH) is the product not only of the causal mutation, but also of modifier genes and environmental factors. Modifier genes are neither necessary nor sufficient to cause HCM, but may significantly affect disease severity (199). The magnitude of effect that modifier genes have on morphologic expression has not yet been systematically explored, but it can be inferred from the phenotypic variability of affected individuals in the same family carrying identical disease-causing mutations. Despite efforts to identify molecules and genes that modify HCM, there has been only limited success. These analyses investigate whether polymorphisms or single nucleotide variations in a putative candidate-modifying gene affect the severity of the clinical phenotypes. Candidate genes have been selected for study, based on their roles in cardiac growth and remodeling, such as molecules involved in the renin-angiotensin-aldosterone system (RAAS) or growth factors such as transforming growth factor (TGFβ1) and insulin-like growth factors (IGF2) (210-212).

Polymorphisms in the RAAS pathway (angiotensinogen-I converting enzyme [ACE], angiotensin receptor 1 [AGTR1], chymase 1 [CMA], angiotensin I [AGT], and cytochrome P450, polypeptide 2 [CYP11B2]) appear to influence the HCM phenotype, in particular the severity of LVH (213,214). Among patients with the DD-ACE genotype, there was greater LVH than among those with an ID or II genotype. Furthermore, a combined ‘pro-LVH’ profile of 5 RAAS genes was associated with a higher degree of LVH in one particular, founder *MYPBC3*-HCM pedigree (213) and in a large cohort of myofilament-positive patients (214).

In 2008, sex hormone polymorphisms were shown to modify the HCM phenotype (215). Fewer CAG-nucleotide repeats in *AR*-encoded androgen receptor were associated with thicker myocardial walls in male subjects, and male carriers of the A allele in the promoter of *ESR1*-encoded estrogen receptor 1 (SNP rs6915267) exhibited an 11% decrease in LV wall thickness compared with GG-homozygote male subjects. HCM

modifier polymorphisms like these could contribute to the clinical differences observed between men and women with HCM (216,217). The release of the complete human genome sequence and the enormity of variation in individuals show a growing role for modifier genes and the search for effect by genome-wide studies. In 2007, Daw et al. (218) performed the first study of this kind for HCM and they identified multiple loci with suggestive linkage. Effect sizes on LV mass on this cohort of 100 patients ranged from about 8 g shift from 1 locus for the common allele to 90 g shift for another locus' uncommon allele.

Environmental influences that contribute to phenotype variability in HCM include diet, lifestyle, and exercise. To date, no large prospective human studies have examined these factors on the cardiac phenotypes of HCM or clinical course.

Exercise has received considerable attention in relationship to sudden death risk. Recognition of these devastating events in the context of vigorous exercise by HCM patients has led to the universal prohibition of competitive sport participation, given a diagnosis of HCM (11). The well-recognized general benefits of exercise for cardiovascular health have prompted more recent evaluation whether modest exercise might be favorable in HCM as well (127). HCM models have afforded a rigorous approach to assessing environmental factors on disease expression. Modest exercise (voluntary cage wheel running) by a mouse model of human HCM showed that exercise delayed onset and development of the hypertrophic phenotype (219). Myocardial hypertrophy, but not cardiac fibrosis, could be partially reversed by voluntary exercise initiated after clinical disease was established. In contrast to these benefits from exercise, highly strenuous enforced exercise in another mouse model of HCM resulted in exaggerated disease expression (192). Diet may also affect disease in mouse models of HCM. Investigators observed a significant reduction in pathologic hypertrophy in response to variable content of soy in diets (220). Severe HCM histopathology, hypertrophy, and fibrosis resulted in mice fed a soy-based diet while mice fed casein (milk) protein-based chow had much less hypertrophy. The relevance of these factors in human disease is yet to be determined.

AIM OF THE STUDY

Aim of this PhD thesis was to identify pathogenic mutations in the most common HCM related genes and to correlate molecular defect with clinical-morphological phenotypic pattern in a large cohort of patients from Padua University Cardiology Clinic.

Since the mutation in the index case has been identified, a cascade screening was performed in the first degree family members searching for affected and unaffected carriers.

METHODS

Population and study design

Since 1980 450 patients with HCM have been referred to the HCM-dedicated outpatients Unit of Cardiology Clinic of Padua University.

Patients came mainly from Veneto region but were also referred from other regions of Italy and most of them were of Caucasian origin.

Diagnosis of HCM was established with demonstration of left ventricular hypertrophy ≥ 15 mm in one or more segments, in the absence of another cardiac or systemic disease capable of producing the magnitude of hypertrophy evident, by two-dimensional echocardiography or CMR.

All index cases and their available family members clinically affected and unaffected, underwent complete cardiological evaluation, including detailed family and personal medical history, physical examination, 12-lead resting ECG, echocardiogram and 24-hours Holter monitoring.

In 200 HCM affected patients (probands or index cases) and 500 relatives (family members), after written informed consent, a 10 ml peripheral blood sample was obtained for DNA analysis.

"Stepwise" genetic testing was designed as reported in the flow-chart in Figure 5. Screening for the 4 most commonly HCM-related sarcomeric genes (i.e. *MYBPC3*, *MYH7*, *TNNT2*, *TNNI3*) was performed by denaturing high performance liquid chromatography (DHPLC) and direct sequencing in 83 HCM index-cases, and a smaller group of 30 probands underwent more extensive mutation screening by 12 genes array-based DNA resequencing assay. Including both screening methods, our population consisted of 99 index cases.

Since one or more pathogenic mutations were found in the proband the same mutation(s) was searched in the first degree relatives in order to identify carriers and plan clinical follow-up.

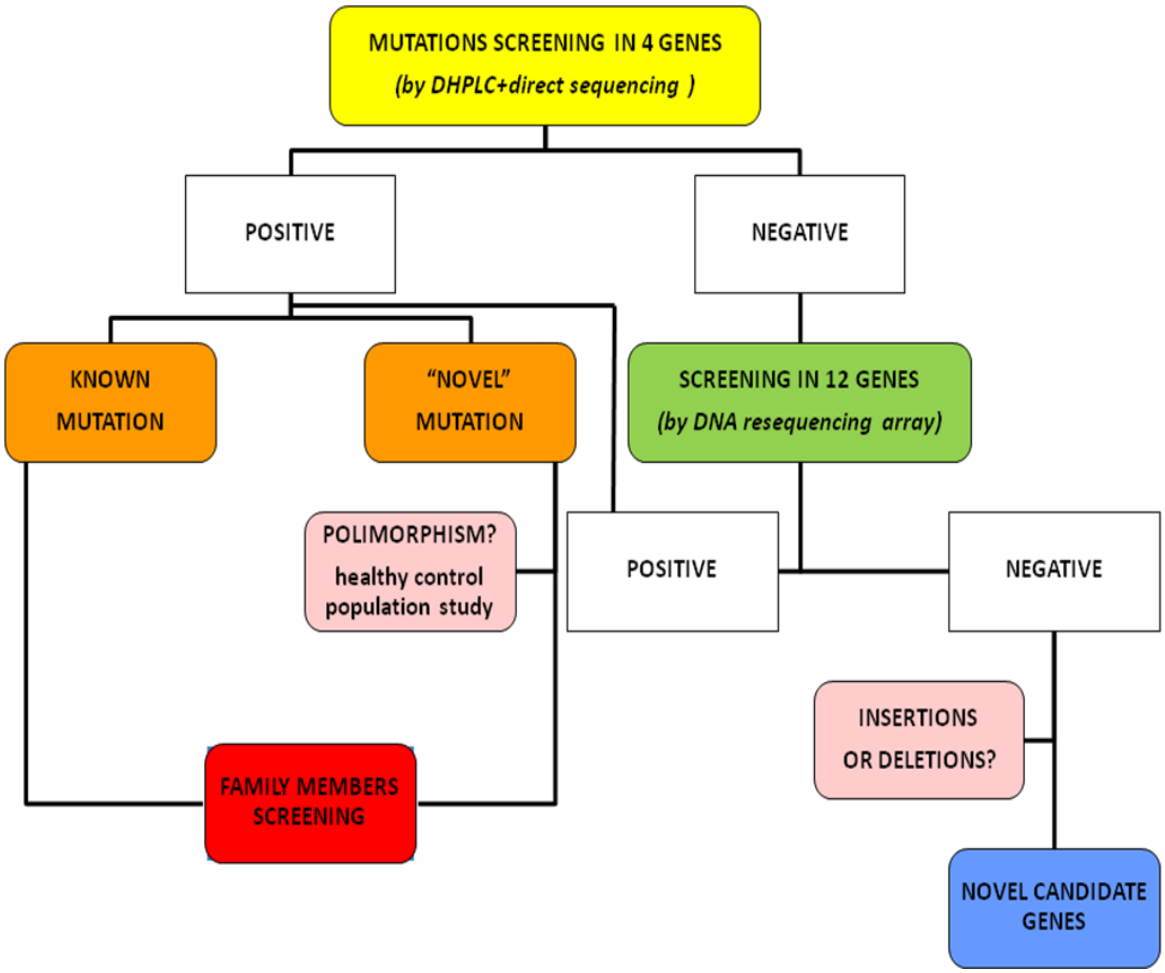

Figure 5. Flow-chart showing genetic screening study design. Probands were initially screened for mutations in the 4 most commonly HCM-related sarcomeric genes (i.e. *MYH7*, *MYBPC3*, *TNNT2* and *TNNI3*) by denaturing high performance liquid chromatography (DHPLC) and direct sequencing. If this analysis was inconclusive they were screened for mutations in 12 (sarcomeric and non-sarcomeric) genes (i.e. *MYH7*, *MYBPC3*, *MYL2*, *MYL3*, *TNNT2*, *TNNI3*, *TNNC1*, *TPM1*, *ACTC*, *CSRP3*, *PLN* and *PRKAG2*) by DNA resequencing array, and finally, in presence of negative results, novel candidate genes were analysed. If the mutations found were novel, these were searched in a healthy control population to rule out the possibility that they represent single nucleotide polymorphisms (SNPs) without a real pathogenetic role in HCM. Once a pathogenic mutation was found in the proband, genetic analysis goes on through the family searching for the same variant in first degree relatives.

Genetic analysis

DNA analysis of MYH7, MYBPC3, TNNT2 and TNNI3 genes by DHPLC and direct sequencing

DNA was extracted from peripheral blood using *salting out* method (221). *In vitro* amplification of *MYH7*, *MYBPC3*, *TNNI3* and *TNNT2* HCM-candidate exons was performed by polymerase chain reaction (PCR) using specific primers, design about 50 bp upstream and downstream of each exon. The DNA analysis were performed by denaturing high-performance liquid chromatography (DHPLC) (222) and some particular exons were analyzed by direct sequencing. When an abnormal DHPLC elution profile was detected, a new PCR of the same sample was sequenced to identify the sequence variation. DHPLC analysis was performed on a Transgenomic Wave DNA Fragment Analysis System (Model 3500HT; Transgenomic) with a DNASep column (Transgenomic). PCR products were denatured for 5 min at 95°C and then left to reanneal slowly at room temperature to promote the formation of heteroduplex. Column temperatures for each amplicons analysis were calculated by NAVIGATOR software (Transgenomic). Whenever fragments showed different melting domains, additional analyzing temperatures were used, to optimize resolution. These temperatures should give the 75-80% of double strand DNA within the fragments. Patient analysis was performed using an application called Mutation Detection (8 minutes for run) at a flow rate of 0.9ml/min, whereas for healthy subjects analysis was used a Rapid DNA application (3 minutes for run) at a flow rate of 1.5ml/min. The acetonitrile gradient was adjusted to elute the DNA at half run, around 4 minutes for Mutation Detection application and 1.5 minutes for Rapid DNA application. The gradient was obtained by mixing Buffer A (0.1M TEAA, pH 7.0) and Buffer B (0.1M TEAA, pH 7.0, 25% acetonitrile). Buffer B increase was 2% per min (with flow rate 0.9 ml/min) and 5% per min (with flow rate 1.5 ml/min).

This analysis was performed by Geneticists of Padua University Biology Department (Prof. Gian Antonio Danieli, Prof. Alessandra Rampazzo, Dr. Marzia De Bortoli, Dr. Gessica Smaniotto).

Novel mutations were considered as disease-causing only if they were absent in 300 unrelated chromosomes from adult, ethnically matched, healthy control participants and they produced a change in a highly conserved amino acid or nucleotide among different species and isoforms.

DNA analysis of 12 sarcomeric and non-sarcomeric genes by DNA resequencing array

In a subgroup of 30 HCM patients we decided to go on with genetic analysis and started a collaboration with Dr. Siv Fokstuen team of Genetic Medicine Laboratory from University of Geneva, Switzerland. In order to overcome the laborious and expensive approach of conventional mutation screening, they have developed a 27 kbp custom-DNA-resequencing array for 12 HCM candidate genes (223,224). Resequencing microarrays consist of a high density of oligonucleotide probes synthesized on the array by photolithography and solid-phase DNA synthesis for hybridization-based analysis of specific sequences of interest (225-227). For each position of the interrogated sequence, eight probes are represented on the array: four probes for each strand, each with a different nucleotide in the middle (A,G,C,T), one perfect match for the reference sequence and three mismatches, allowing the detection of all possible nucleotide substitutions of both strands (Figure 6).

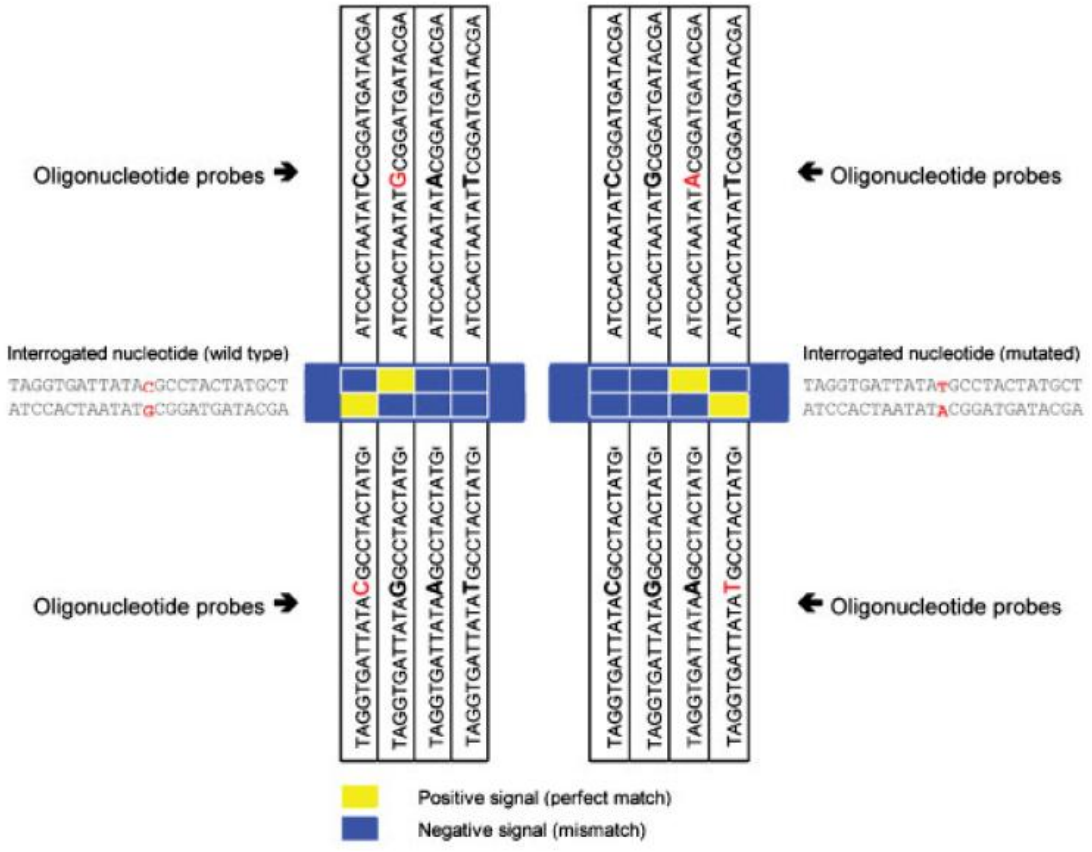

Figure 6. Principle of the array. For each double-stranded nucleotide position interrogated (resequenced), there are eight oligonucleotides placed on the array-two perfect matches and six mismatches-thus all possible combinations are present for each nucleotide. After hybridization with the PCR products of the patients DNA the sequence can be determined at each position. From Fokstuen et al *Hum Mutat 2008; 29:879-85 (223).*

Fokstuen et al. designed a resequencing array based on commercially available 30 kbp customSEQ (Affymetrix, Santa Clara, CA) with reference sequences of 12 HCM genes: *MYH7, MYBPC3, TNNT2, TPM1, TNNI3, MYL3, MYL2, CSRP3, PLN, ACTC, TNNC1*, and *PRKAG2*. Based on the ENSEMBL database (www.ensembl.org/Homo_sapiens/index.html) the forward and reverse strands of all coding exons (a total of 160), the acceptor and donor splice sites (20 bp beyond the splice junctions), the characterized or annotated promoter sequences (known for genes *MYL3, MYL2, TNNC1, PRKAG2, TNNI3*), the 3 noncoding exons (two for *MYH7* and one for *PLN*), or a minimum of 175 bp of sequence upstream to the first known exon were placed on the array. In total 29124 features are tiled on the 30-kbp array and 25260 bp of double-stranded gene sequences are analyzed. The remaining oligonucleotides represent control DNA (Affymetrix control reference sequence; AFFX-TagIQ-EX) and technical constraints (12 bp at both ends of each exon).

The various steps of the array experiment are here briefly summarized: quantified pooled DNA is fragmented, end-labeled with biotin, prehybridized and hybridized to the array for 16 hr at 45°C. Staining and stringency washing are performed on automated fluidic stations. Hybridization signals were read by a high resolution laser scanner and interpretation of the sequences was carried out by the GeneChip DNA Analysis Software (GDAS, vers. 2.0; Affymetrix) and by the GeneChip Resequencing Analysis Software (GSEQ, vers.4.0; Affymetrix), which align the sequence of several samples on the resequencing window, along with the reference sequence. Heterozygous or homozygous single nucleotide substitutions are shown in International Union of Pure and Allied Chemistry (IUPAC) code and are color-coded. All nonsynonymous nucleotide changes are subsequently validated by direct sequencing of the appropriate PCR product.

Novel disease-candidate genes

If also DNA resequencing array analysis was inconclusive, DNA samples have been sent to Dr. Andreas Perrot, Charitè University of Berlin, Germany, in order to search presence of HCM-causing mutations in novel candidate genes, particularly genes coding for Z-disk proteins (228,229).

Clinical evaluation

All index cases and, when possible, their relatives underwent complete cardiological evaluation including family and personal medical history, physical examination, 12-lead resting ECG, echocardiogram and 24-hours Holter monitoring.

For probands and affected family members clinic follow-up controls were planned every 6-12 months, whereas unaffected relatives underwent periodic screening at intervals of every 12 months during adolescence and every 5 years in adults as well as at the onset of symptoms suggestive of HCM.

Anamnesis (family history) attempted to reconstruct family tree, identify presence of cardiac diseases or sudden cardiac death in relatives, show inheritance modality, and collect symptoms suggestive of HCM as reduced exercise capacity, chest pain, syncope, and other data useful in risk stratification.

A scrupulous physical examination was performed in order to find out typical signs of the disease as systolic heart murmur that characteristically increases with Valsalva manoeuvre suggestive of obstructive HCM, as well as heart failure signs (pulmonary congestion, jugular or limb swelling), indicative of hemodynamic deterioration.

Resting 12-leads ECG raised or confirmed diagnostic suspicious of HCM and were informative about rhythm, LV hypertrophy, conduction disturbances, whereas 24-hrs dynamic Holter monitoring documented presence and frequency of arrhythmic events (i.e. supraventricular arrhythmias, ectopic beats, sustained or non sustained ventricular tachycardia).

A complete echocardiographic evaluation (including M-mode, 2D, continuous and pulsed-wave Doppler as well as TDI) was mandatory for HCM diagnosis, classification (obstructive or not) and phenotype characterization and was performed in all probands and relatives.

In selected subgroups of patients, on the basis of diagnostic question and clinical status, also additional diagnostic exams were performed, i.e. contrast-enhanced CMR, exercise testing, cardiac computed tomography, coronary angiography, SPECT, and endocardial biopsy.

Pathological examination

Gross and histopathological features were analysed in patients for whom the heart was available for inspection (from autopsy or explanted hearts). Gross examination was

performed by expert cardiovascular Pathologists (Prof. Gaetano Thiene, Prof. Annalisa Angelini, Prof. Cristina Basso) and addressed heart weight, LV wall thickness (i.e. exclusive papillary muscles and trabeculae), chamber dilatation, coronary arterial anatomy, and scarring. Tissue specimens were embedded in paraffin, sectioned at 6 mm and stained with haematoxylin–eosin and Azan-Heidenhein trichrome. Blocks were examined microscopically to assess myocyte disarray, interstitial and replacement fibrosis, and intramural small vessel disease.

Statistical analysis

Data are expressed as frequencies or mean ± SD for continuous variables. Differences between means were tested by unpaired Student's *t*-test. Categorical frequencies were compared by chi-square or Fisher's exact test, where appropriate. Probability values reported are 2-sided, and values < 0.05 were considered statistically significant. Survival curves were estimated by the Kaplan-Meier method and compared by the log-rank test. SPSS statistics 17.0 (SPSS Inc., Chicago, IL, USA) was used for analysis.

RESULTS

Patients population

Including both screening methods, our population consisted of 99 index cases.

Sixty-four of them (65%) were probands belonging to families with several affected members ("familial cases") whereas the remaining 35 (35%) had not family history of HCM, therefore were considered as "sporadic cases".

Population characteristics and comparison between familial and sporadic cases are summarized in Table 5.

Most of patients were males (70, 71%). Age at diagnosis was in mean 31 ± 17 years (median 30 years, range 1-77 years) and age at last control was 45 ± 17 years (median 47 years, range 9-77 years), with a mean follow-up of 9 ± 8 years (range 0-28 years). HCM was mainly diagnosed for presence of symptoms (49%) and it was occasional (i.e. for ECG abnormalities or cardiac murmur) in 33% of patients. In a small cohort of patients HCM was suspected at preparticipation screening of competitive athletes (5%) or familial screening (7%).

Obstructive HCM forms were about one-third of cases (38%) and MLVWT ranged from 15 to 46 mm (mean 25 ± 7 mm, median 25 mm). In the majority of patients LV EF was normal (mean 57 ± 10%), whereas EF <50% (defined as "end-stage" phase) was detected in 14% of probands.

About one-fifth of patients (22%) were asymptomatic, 41% were in NYHA class I, 27% in class II, 19% in class III and 7% in class IV. One-fourth (25%) had at least one syncope. Atrial fibrillation complicated the clinical course in one-fourth of patients and it was paroxysmal in 36% of cases, persistent in 14%, permanent in 45% and the date of onset was unknown in 5%.

No clinically relevant statistically significant differences were found between familial and sporadic cases.

Table 5. Population characteristics and comparison between familial and sporadic cases.

	All index cases (n=99)	**Familial cases (n=64)**	**Sporadic cases (n=35)**	**p (familial vs sporadic cases)**
Male, n (%)	70 (71)	43 (67)	27 (77)	0.29
Female, n (%)	29 (29)	21 (33)	8 (23)	0.29
Age at diagnosis, years				
range	1-77	1-69	3-77	
mean	31 ± 17	30 ± 16	34 ± 19	0.34
Diagnosis for, n (%)				
a. symptoms	48 (49)	30 (47)	18 (51)	0.66
b. family screening	7 (7)	7 (11)	0	0.04
c. athletic screening	5 (5)	5 (8)	0	0.09
d. occasional	33 (33)	17 (26)	16 (46)	0.05
e. unknown	6 (6)	5 (8)	1 (3)	0.3
Age at last control, years	45 ± 17	44 ± 17	45 ± 17	0.74
Duration of follow-up, years	9 ± 8	9 ± 8	9 ± 8	0.32
Obstructive forms, n (%)	38 (38)	23 (36)	15 (43)	0.49
MLVWT, mm				
range	15-46	15-37	15-46	
mean	25 ± 7	24 ± 7	26 ± 8	0.61
EF, (%)	57 ± 10	57 ± 10	58 ± 12	0.82
End-stage patients (EF<50%), n (%)	14 (14)	9 (14)	5 (14)	0.97
Asymptomatic pts, n (%)	22 (22)	12 (19)	10 (28)	0.26
NYHA class, n (%)				
I	40 (41)	24 (38)	16(45)	0.42
II	27 (27)	22 (34)	5 (14)	0.03
III	19 (19)	11 (17)	8 (23)	0.49
IV	7 (7)	4 (6)	3 (9)	0.66
Unknown	6 (6)	3 (5)	3 (9)	0.43
Syncope, n (%)	25 (25)	20 (31)	5 (14)	0.06
AF, n (%)	22 (22)	14 (22)	8 (23)	0.91
a. Paroxysmal	8 (36)	6 (43)	2 (25)	0.40
b. Persistent	3 (14)	2 (14)	1 (12)	0.90
c. Permanent	10 (45)	5 (36)	5 (63)	0.22
d. Unknown	1 (5)	1 (7)	0	0.43

AF, atrial fibrillation; EF, ejection fraction; MLVWT, maximal left ventricular wall thickness.

Mutations

DNA analysis of the 4 most commonly HCM-related sarcomeric genes (i.e. *MYH7*, *MYBPC3*, *TNNT2* and *TNNI3*) by DHPLC and direct sequencing revealed presence of 22 different pathogenic mutations in 25/83 (30%) index cases.

In two cases the same mutation was found in more probands (an identical *MYH7* mutation - Gly716Arg in exon 19 - was present in 2 patients and the same *MYBPC3* mutation - Ala364Thr in exon 11 - in 5 index cases from different families and geographical regions).

Two patients had multiple (double) mutations in a compound heterozygous state.

There were no difference in frequency of mutations between probands with family history of HCM (or familial cases) and sporadic cases (16/53, 30% vs 9/30, 30%, p=0.98).

Nineteen mutations were localized in exons, whereas 3 variants were intronic but very close to the coding regions with a probable role in RNA splicing.

Nine mutations were already described in literature as HCM-causing, while the remaining 13 were "novel", but not found in a healthy control population of unrelated 300 chromosomes, so they do not represent SNPs.

MYH7 gene mutations

The most frequently HCM-related gene was *MYH7* with 10 (of 22, 45%) mutations found in 10 patients (Table 6).

Table 6. Mutations in *MYH7* gene.

Gene	Amplicon	Nucleotide change	Aminoacid change	Reference (n)	Index cases (n)
MHY7	5	c.428G>A	p.Arg143Gln	Kimura et al (230)	#75
MYH7	9	c.746G>T	p.Arg249Leu	Novel	#17
MHY7	13	c.1219G>T	p.Gly407Cys	Melacini et al (55)	#59
MHY7	19	c.2146G>A	p.Gly716Arg	Anan et al (231)	#6 #12*
MHY7	19	c.2156G>A	p.Arg719Gln	Consevage et al (232)	#2
MHY7	22	c.2631G>T	p.Met877Ile	Melacini et al (55)	#57
MHY7	27	c.3621C>G	p.Ile1207Met	Melacini et al (108)	#13
MYH7	35	c.4962G>T	p.Gln1654Hys	Novel	#12*
MYH7	36	c.5279C>T	p.Thr1760Met	Fokstuen et al (223)	#83
MYH7	39	c.5790+7C>T		Novel	#58

* indicates patients with double mutations

Phenotypic expression and clinical course of patients with *MYH7* mutations are shown in Table 7.

Table 7. Phenotypic characteristics and clinical course of patients with *MYH7* mutations.

Pts n.	Gender	Age at dg	HCM/ SD FH	NYHA	MLVWT	LVOT peak gradient	EF (%)	NSVT	AF	Outcome
2	F	20	yes/no	IV	18	n.o.	68	no	yes	Tx (38yrs)
6	F	28	no/no	I	28	n.o.	68	no	no	Alive (33yrs)
12*	F	10	yes/no	I	33	n.o.	66	no	no	SD (17yrs)
13	M	49	no/no	IV	18	n.o.	57	yes	yes	Tx (60yrs)
17	M	17	no/no	IV	16	n.o.	35	no	yes	HF death (24yrs)
57	M	16	yes/no	II	32	n.o.	74	yes	no	Alive(40yrs) ICD
58	M	39	no/no	II	27	85	57	no	no	Alive (53yrs)
59	M	16	yes/no	I	20	n.o.	54	no	no	SD (24yrs)
75	M	25	yes/yes	I	17	n.o.	57	no	no	Alive (52yrs)
83	F	47	yes/yes	III	17	n.o.	54	no	yes	HF(58yrs) PM

AF, atrial fibrillation; dg, diagnosis; EF, ejection fraction; FH, family history; HF, heart failure; HCM, hypertrophic cardiomyopathy; ICD, implantable cardioverter defibrillator; MLVWT, maximal left ventricular wall thickness; LVOT, left ventricular outflow tract; n.o., non obstructive; NSVT, non sustained ventricular tachycardia; PM, pacemaker; SD, sudden death; Tx, cardiac transplant; * indicates patients with double mutations

The same *MYH7* mutation (Gly716Arg) was present in 2 index cases (#6 and #12) from different families, but in one of them (#12) it was associated with a second *MYH7* mutation (Gln1654Hys), in a compound heterozygous state, and this proband, with a massive LVH, died suddenly at young age (17 years).

Another sudden death occurred in a young man (#59) with a *MYH7* mutation (Gly407Cys), focal fibrosis at CMR, but without any other known risk factor (Figure 7).

A lady from a large HCM-family (#83) with *MHY7* Thr1760Met mutation underwent severe and progressive heart failure only partially responsive to pharmacological therapy, whereas another 24 years old proband (#17) with *MHY7* Arg249Leu mutation died of refractory heart failure.

Moreover, two other index cases (#2 and #13) with mutations in *MYH7* underwent cardiac transplants at 38 and 60 years respectively.

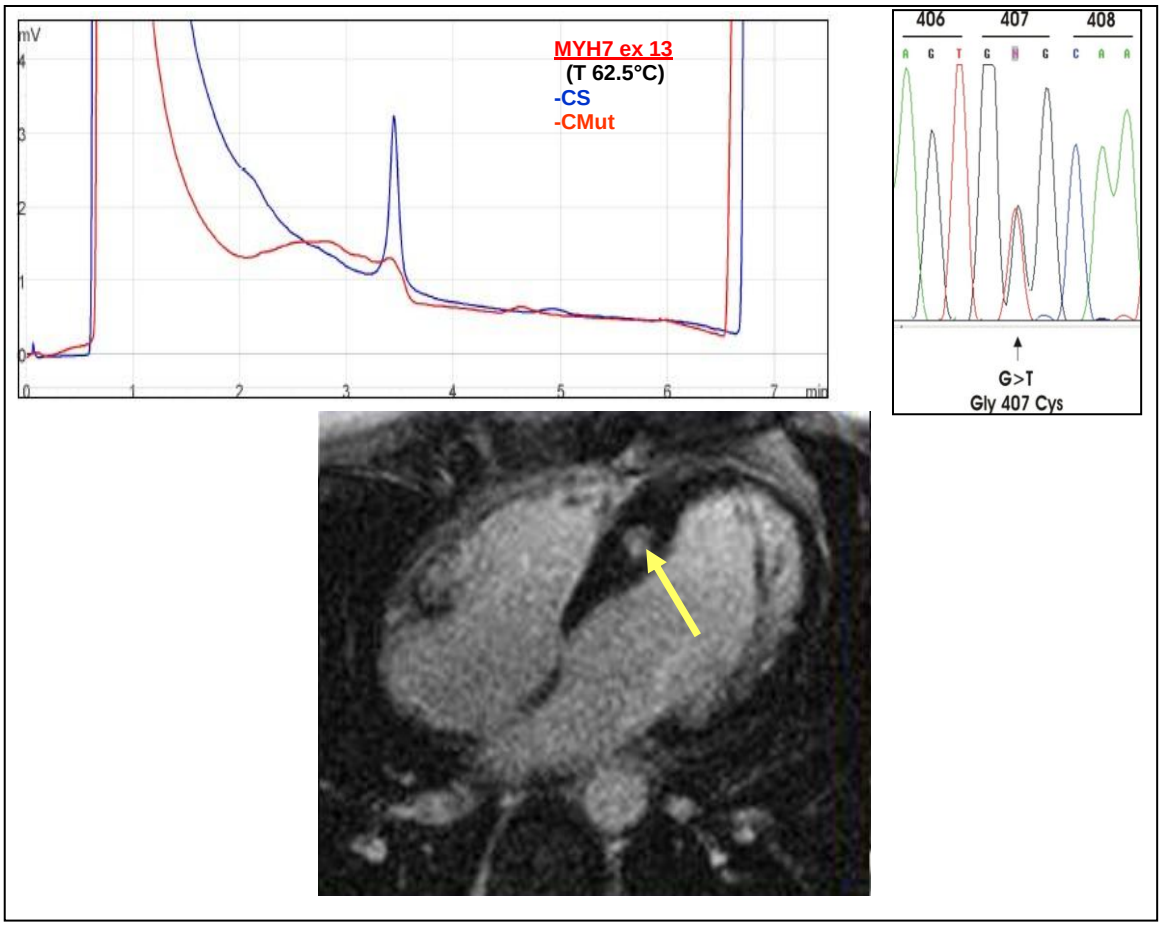

Figure 7. DHPLC elution profile, direct sequencing and contrast-enhanced CMR of patient #59. A mutation of *MYH7* (exon 13, position 407) and a focal scarring at gadolinium enhanced-CMR (arrow) indicative of myocardial fibrosis are shown in this young patient who died suddenly when he was 24 years old.

MYBPC3 gene mutations

The second gene most frequently involved in HCM pathogenesis was *MYBPC3* encoding cardiac myosin-binding protein C, with 6 different mutations (27% of total mutations) in 10 patients (Table 8).

Table 8. Mutations in *MYBPC3* gene.

Gene	Amplicon	Nucleotide change	Aminoacid change	Reference (n)	Index cases (n)
MYBPC3	11	c.1090G>A	p.Ala364Thr	Melacini et al (108)	#29 #30 #37 #48 #74
MYBPC3	12	c.1112C>G	p.Pro371Arg	Girolami et al (233)	#49
MYBPC3	12	c.1128C>T	p.Gln366X	Melacini et al (108)	#20
MYBPC3	23	c.2429G>A	p.Arg810Hys	Nanni et al (234)	#61
MYBPC3	23	c.2544C>A	p.Ala848Glu	Novel	#81
MYBPC3	5	c.538-2A>C		Novel	#3

Table 9 shows characteristics and clinical course of patients with *MYH7* mutations.

Table 9. Phenotypic characteristics and clinical course of patients with *MYBPC3* mutations.

Pts n.	Gender	Age at dg	HCM/ SD FH	NYHA	MLVWT	LVOT peak gradient	EF (%)	VT	AF	Outcome
3	M	28	yes/no	I	28	n.o.	70	no	no	Alive (39yrs)
20	F	59	no/no	III	23	n.o.	31	SVT	no	Tx (63yrs)
29	M	12	no/no	I	36	46	62	NSVT	no	SD (22yrs)
30*	M	35	no/no	I	27	73	72	no	no	Alive (51)
37	F	1	yes/no	IV	30	n.o.	82	no	no	Tx (12yrs), death for reject (20yrs)
48	M	69	yes/yes	II	31	n.o.	55	no	no	Alive (75)
49	M	18	yes/yes	I	30	n.o.	57	no	no	Alive (23yrs) ICD
61	M	3	no/no	I	34	n.o.	55	no	no	Alive (16yrs)
74	M	42	yes/no	II	30	n.o.	62	NSVT	no	Alive (66yrs) ICD
81	M	37	no/no	II	36	92	59	no	no	Alive (41yrs)

AF, atrial fibrillation; dg, diagnosis; EF, ejection fraction; FH, family history; HF, heart failure; HCM, hypertrophic cardiomyopathy; ICD, implantable cardioverter defibrillator; MLVWT, maximal left ventricular wall thickness; LVOT, left ventricular outflow tract; n.o., non obstructive; NSVT, non sustained ventricular tachycardia; PM, pacemaker; SD, sudden death; SVT, sustained ventricular tachycardia; Tx, cardiac transplant; VT, ventricular tachycardia; *indicates patients with double mutations.

The same *MYBPC3* mutation - Ala364Thr in exon 11- was detected in 5 index cases (#29, #30, #37, #48, #74) belonging to different families from different regions. This was a novel mutation that was not found in 300 healthy control chromosomes and therefore do not represent a common polymorphism. All these index cases had severe or massive HCM phenotype; one of them (#29) died suddenly when he was 22 years old, and a young female (#37) underwent cardiac transplant at 12 years of age and 8 years later she died for rejection (Figure 8).

Another proband with *MYBPC3* (#20) mutation had an "end-stage" form of HCM with severe systolic dysfunction and progressive heart failure, that required cardiac transplant.

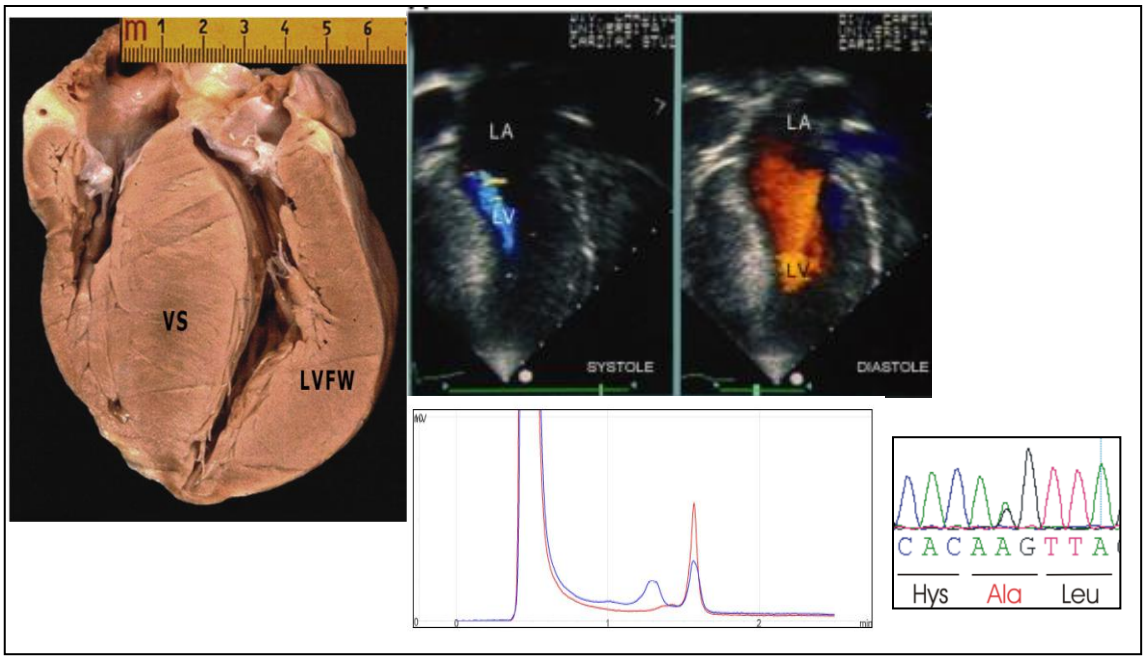

Figure 8. Explanted heart, echo with color-Doppler, DHPLC elution profile and direct sequencing of patient #37. A mutation of *MYBPC3* (exon 11, position 364) is responsible for a massive left ventricular hypertrophy in this female child who underwent cardiac transplant. Published in Melacini et al. *Eur Heart J* 2010; 31:2111–2123 (108).

TNNT2 and TNNI3 genes mutations

Mutations in genes coding cardiac troponin T and I (*TNNT2* and *TNNI3* respectively) were found less frequently, in particular 2 different (9%) mutations of *TNNT2* were found in 2 probands and 4 (18%) mutations of *TNNI3* in 4 probands (Table 9) whose characteristics and clinical course are summarized in Table 10.

Table 9. Mutations in *TNNT2 and TNNI3* genes.

Gene	Amplicon	Nucleotide change	Aminoacid change	Reference (n)	Index cases (n)
TNNT2	9	c.274C>T	p.Arg92Trp	Koga et al (235)	#40
TNNT2	9	c.281G>T	p.Arg94Leu	Varnava et al (236)	#5
TNNI3	8	c.557G>A	p.Arg186Gln	Richard et al (180)	#66
TNNI3	8	c.563T>C	p.Val188Ala	Novel	#51
TNNI3	8	c.620A>C	p.Lys207Thr	Melacini et al (108)	#65
TNNI3	5	c.371+6G>A		Novel	#30

Table 10. Phenotypic characteristics and clinical course of patients with *TNNT2* and *TNNI3* mutations.

Pts n.	Gene	gender	Age at dg	HCM/ SD FH	NYHA	MLVWT	LVOT peak gradient	EF (%)	VT	AF	Outcome
5	*TNNT2*	M	40	no/no	III	15	n.o.	30	no	yes	Tx (59yrs)
40	*TNNT2*	M	38	yes/no	I	16	n.o.	41	no	no	HFD(53yrs)
30*	*TNNI3*	M	35	no/no	I	27	73	72	no	no	Alive (51yrs)
51	*TNNI3*	F	44	yes/no	II	18	n.o.	55	no	yes	HF, alive (64yrs)
65	*TNNI3*	F	28	yes/no	III	17	n.o.	59	no	no	Tx (29yrs)
66	*TNNI3*	F	21	yes/no	II	26	n.o.	62	FV	no	aSD(21yrs), ICD

AF, atrial fibrillation; aSD, aborted sudden death; dg, diagnosis; EF, ejection fraction; FH, family history; HF, heart failure; HFD, heart failure death; HCM, hypertrophic cardiomyopathy; ICD, implantable cardioverter defibrillator; MLVWT, maximal left ventricular wall thickness; LVOT, left ventricular outflow tract; n.o., non obstructive; SD, sudden death; Tx, cardiac transplant; VT, ventricular tachycardia; * indicates patients with double mutations.

Mutations in troponin T gene were associated with mild hypertrophy, systolic dysfunction, restrictive LV filling pattern and severe clinical course: the former index case (#40) died of heart failure and the latter underwent cardiac transplant (#5, Figure 9).

Among patients with mutations in cardiac troponin I, a young (21 years) female proband (#66) was resuscitated from ventricular fibrillation and received an ICD for secondary prevention, another young patient (#65) underwent cardiac transplant for drug-refractory heart failure when she was 29 years old, and proband #51 experienced severe heart failure worsened by permanent AF.

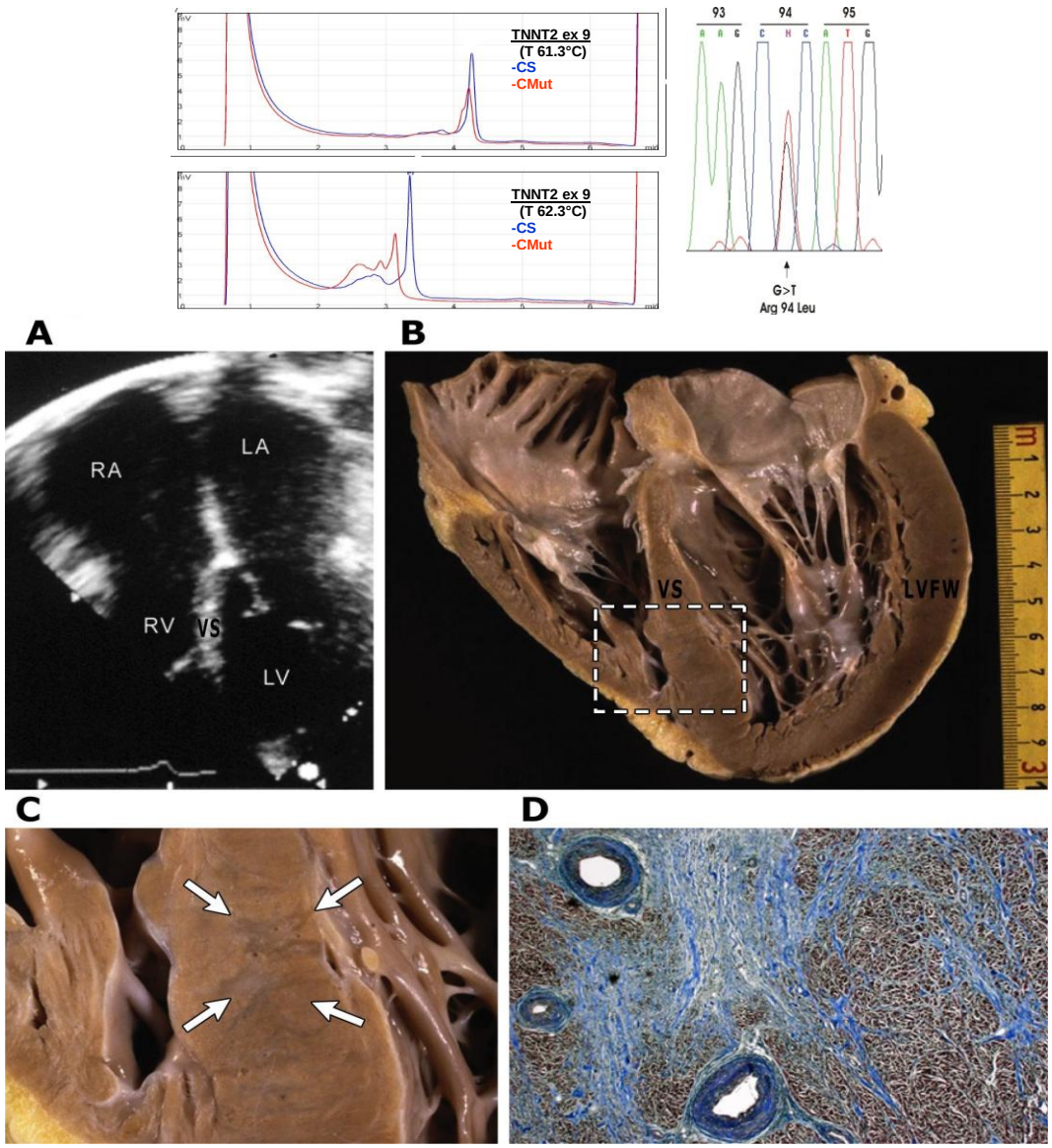

Figure 9. DHPLC elution profile, direct sequencing, echo, explanted heart (macroscopic specimen and histology) of patient #5. A mutation in *TNNT2* gene was found in this patient with mild hypertrophy, systolic dysfunction and restrictive LV filling pattern, who underwent cardiac transplant. (A) Four-chamber view at end-diastole showing dilatation of both atria, left ventricular (LV) enlargement, and mild hypertrophy; ejection fraction was 30%. (B) Heart removed at transplantation. Note thinning of basal and mid-ventricular septum (VS) compared with distal LV. (C) High power of boxed area in (B). Greyish areas (arrows) are indicative of septal scarring. (D) Area of septum shown in (C): extensive replacement fibrosis is associated with abnormal intramural arterioles. (Trichrome stain × 60). LA, left atrium; RA, right atrium; RV, right ventricle. Published in Melacini et al. *Eur Heart J* 2010; 31:2111–2123 (108).

Since screening of mutations in 4 sarcomeric genes by DHPLC and direct sequencing was inconclusive in more than two-third (70%) of probands, we decided to go on with genetic analysis. A subgroup of 14 DNA samples from patients who were previously screened by DHPLC and 16 from other index cases not yet analyzed, were sent to Genetic Medicine Laboratory of Geneva University for DNA resequencing array analysis of 12 HCM candidate genes.

By DNA resequencing array analysis were found 7 mutations in 8/30 probands (27%) as described in Table 11, 12, 13 and 14.

Three different mutation of *MYBPC3* gene were found in 4 probands, and one in *MYH7, TNNT2, TNNI3* and *MYL3* (coding for essential myosin light chain) genes respectively. Ala364Thr mutation in exon 11 of *MYBPC3* as well as Arg186Gln in exon 8 of *TNNI3* were detected in the same patients with both genetic screening methods (Table 12). No mutations found by DHPLC were missed by DNA resequencing method except for an intronic substitution in *TNNI3* (371+6G>A in patient #30). The remaining mutations found by resequencing array were in probands not previously screened by DHPLC (Table 13).

Table 11. Results of DNA resequencing array analysis for mutations screening of 12 genes in 30 index-cases.

Gene	Amplicon	Nucleotide Change	Aminoacid change	Reference	Index cases (n)
MYH7	25	c.3133C>T	p.Arg1045Cys	Olivotto et al (237)	#92
MYBPC3	11	c.1090G>A	p.Ala364Thr	Melacini et al (108)	#30 #37
MYBPC3	15	c.1624G>C	p.Glu542Gln	Carrier et al (238)	#99
MYBPC3	20	c.2111C>A	p.Thr704Lys	Fokstuen (224)	#98
TNNT2	16	c.832C>T	p.Arg278Cys	Watkins et al (117)	#95
TNNI3	8	c.557G>A	p.Arg186Gln	Richard et al (180)	#66
MYL3	3	c.170C>A	p.A57D	Fokstuen (224)	#88

Table 12. Results of genetic analysis in 14 index cases screened both by DHPLC and by DNA resequencing array.

Gene	Exon	Nucleotide Change	Aminoacid change	Reference	Index cases (n)
MYBPC3	11	c.1090G>A	p.Ala364Thr	Melacini et al (108)	#30* #37
TNNI3	8	c.557G>A	p.Arg186Gln	Richard et al (180)	#66
TNNI3	5	c.371+6G>A		Novel	#30*

* indicates patients with double mutations.

Table 13. Results of genetic analysis in 16 index cases screened only by DNA resequencing array.

Gene	Exon	Nucleotide Change	Aminoacid change	Reference	Index cases (n)
MYH7	25	c.3133C>T	p.Arg1045Cys	Olivotto et al (237)	#92
MYBPC3	15	c.1624G>C	p.Glu542Gln	Carrier et al (238)	#99
MYBPC3	20	c.2114C>A	p.Thr705Lys	Fokstuen et al (224)	#98
TNNT2	16	c.832C>T	p.Arg278Cys	Watkins et al (117)	#95
MYL3	3	c.170C>A	p.Ala57Asp	Fokstuen et al (224)	#88

Table 14. Phenotypic characteristics and clinical course of patients with mutations found by DNA resequencing array.

Pts n.	Gene	gender	Age at dg	HCM/ SD FH	NYHA	Max LVWT	LVOT peak gradient	EF (%)	VT	AF	Outcome
92	*MYH7*	M	36	yes/yes	II	21	n.o.	59	no	no	Alive (41yrs)
30*	*MYBPC3*	M	35	no/no	I	27	73	72	no	no	Alive (51yrs)
37	*MYBPC3*	F	1	yes/no	IV	30	n.o.	82	no	no	Tx (12yrs) death for rejection (20yrs)
98	*MYBPC3*	F	27	yes/no	I	23	n.o.	73	no	no	SD (42yrs)
99	*MYBPC3*	M	38	yes/no	II	27	n.o.	70	NSVT	no	Alive (43yrs)
95	*TNNT2*	F	13	yes/yes	II	27	n.o.	76	no	no	Alive (21yrs), ICD
88	*MYL3*	M	36	yes/yes	I	17	n.o.	58	no	yes	Alive (46yrs)

AF, atrial fibrillation; dg, diagnosis; EF, ejection fraction; FH, family history; HCM, hypertrophic cardiomyopathy; ICD, implantable cardioverter defibrillator; LVWT, left ventricular wall thickness; LVOT, left ventricular outflow tract; n.o., non obstructive; NSVT, non sustained ventricular tachycardia; SD, sudden death; SVT, sustained ventricular tachycardia; Tx, cardiac transplant; VF, ventricular fibrillation; VT, ventricular tachycardia; * indicates patients with double mutations.

Including both DHPLC and DNA resequencing array screening methods, in our 99 index cases population, 27 pathogenic HCM-causing mutations were found in 30 probands (30%).

Genes involved, frequencies of mutations and number of probands are summarized in Table 15.

Table 15. Results of genetic screening.

	n. of mutations found			n. of index cases with mutation(s)		
Gene	**DHPLC**	**DNA array**	**total**	**DHPLC**	**DNA array**	**total**
MYH7	10	1	11	10	1	11
MYBPC3	6	2	8	10	2	12
TNNT2	2	1	3	2	1	3
TNNI3	4	0	4	4	0	4
MYL3	-	1	1	-	1	1
total	**22**	**5**	**27**	**25***	**5**	**30***

* Proband #30 had a double heterozygous mutation in *MYH7* and *TNNI3* genes, so he was count in the total amount of probands with mutation(s) just once.

Polymorphisms (SNPs)

Several different single nucleotide polymorphisms (SNPs), defined as variants of uncertain pathological significance with a frequency higher than 1% in the general population, were found either by DHPLC or DNA resequencing array (Table 16).

Table 16. SNPs found by DNA resequencing array.

Gene	**Exon**	**Nucleotide change**	**Aminoacid change**	**Db SNP rs#**	**note**	**Patient n.**
MHY7	32	c.4578C>G	p.Ser1491Cys	rs3729823		#97
MYBPC3	4	c.527G>A	p.Val158Met	rs3729986		#1
MYBPC3	INTR	IVS4-12DelC		rs11570050	Second SNP	#66
MYBPC3	5	c.620G>A	p.Val189Ile	rs11570052		#30 #41 #59
MYBPC3	6	c.761A>G	p.Ser236Gly	rs3729989		#73 #94
MYBPC3	11	c.977G>A	p.Arg326Gln	rs34580776		#27
TNNT2	15	c.827A>G	p.Lys253Arg	rs3730238		#69 #91

Clinical follow-up

Index cases were clinically followed for 9 ± 8 years and major events were collected. Cardiac deaths, that included 7 sudden deaths, 6 aborted sudden deaths, 4 heart failure deaths and 10 cardiac transplants, occurred in 27 probands, with annual mortality rate of 3%.

Survival curves were estimated by the Kaplan-Meier method in the 83 index cases screened for 4 genes by DHPLC and showed a worse prognosis in probands harbouring

sarcomeric genes mutations compared with probands without mutations (log-rank test p=0.05) as shown in figure 10.

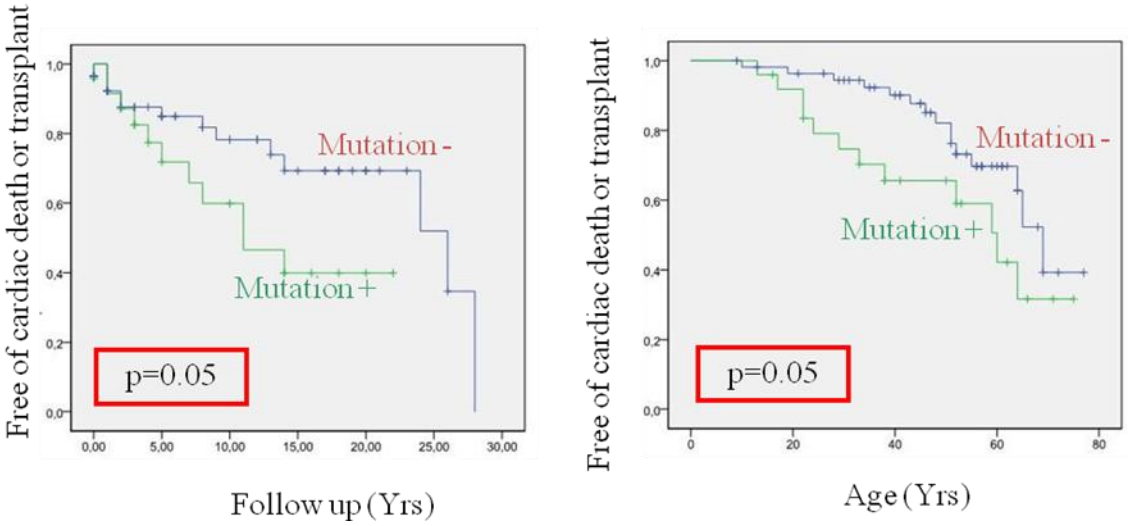

Figure 10. Kaplan-Meier survival curves in probands harbouring mutation (Mutation +) compared with those without mutation (Mutation -) in 4 sarcomeric genes. Event-free survival curves showed a worse prognosis in mutation + probands during clinical follow-up as well as during all life.

Family screening

Since one or more pathogenic mutations have been identified in the index case, genetic analysis went on through the family members to recognize HCM-affected or healthy carriers.

Fifty-one members from 16 different families were screened for the mutation(s) found in their family proband and 23 (45%) resulted carriers.

Eight carriers had phenotypic expression fulfilling diagnostic criteria for HCM, whereas 10 had only minor signs (i.e. mitral prolapse, mild LV hypertrophy with LVWT of 12-13 mm, ECG or TDI abnormalities) suggestive of HCM, but not diagnostic, and 5 were healthy carriers.

Families pedigrees are described in Figures 11-26 and characteristics of carriers (healthy, with evident phenotypic expression of HCM or only minor HCM signs) are summarized in Table 17. Table 18 compares healthy relatives with or without mutation.

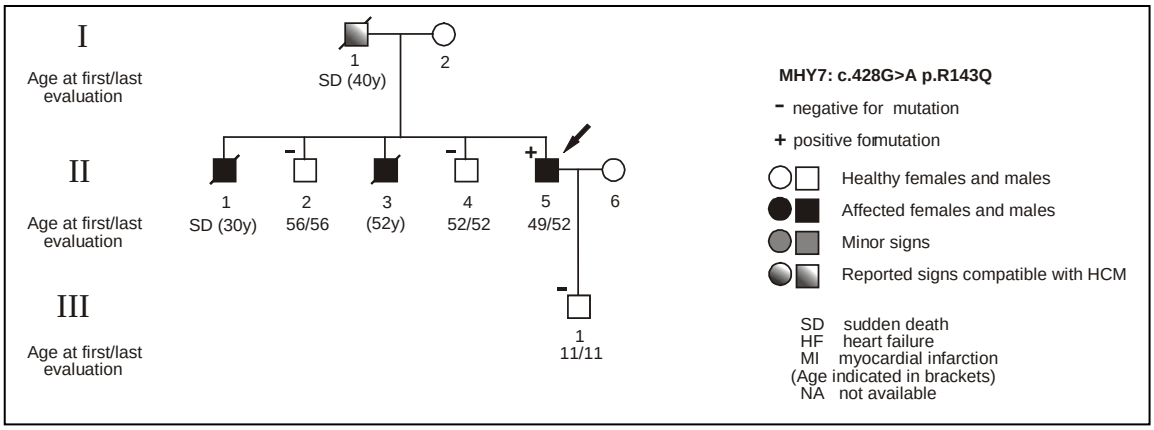

Figure 11. Pedigree of family TO.

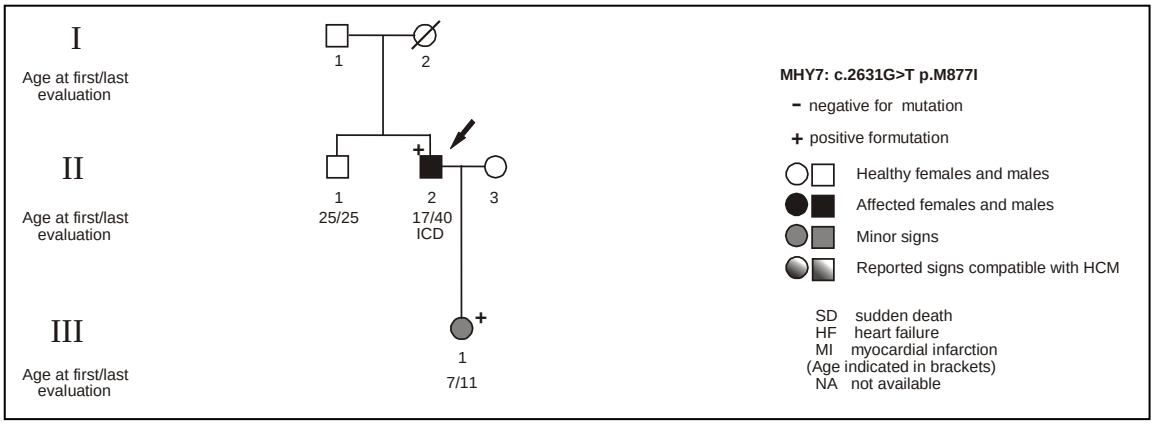

Figure 12. Pedigree of family PA.

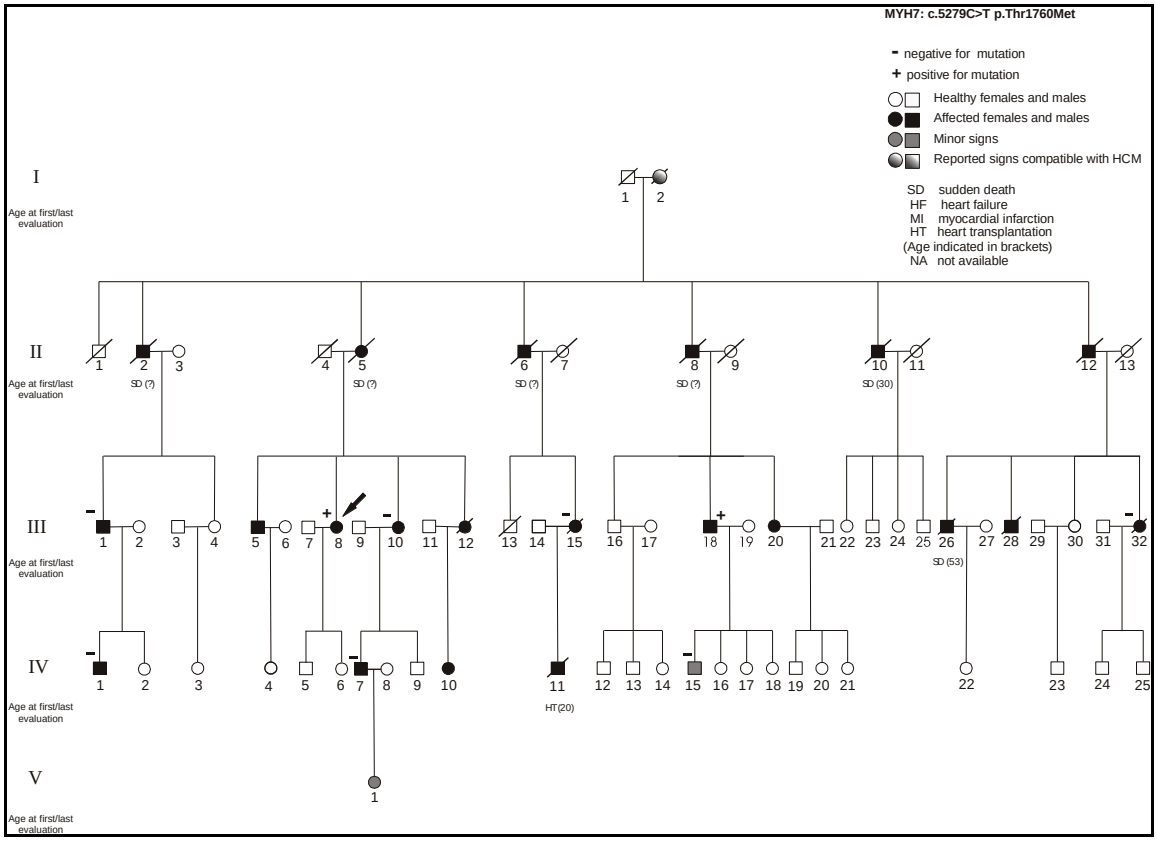

Figure 13. Pedigree of family TZ.

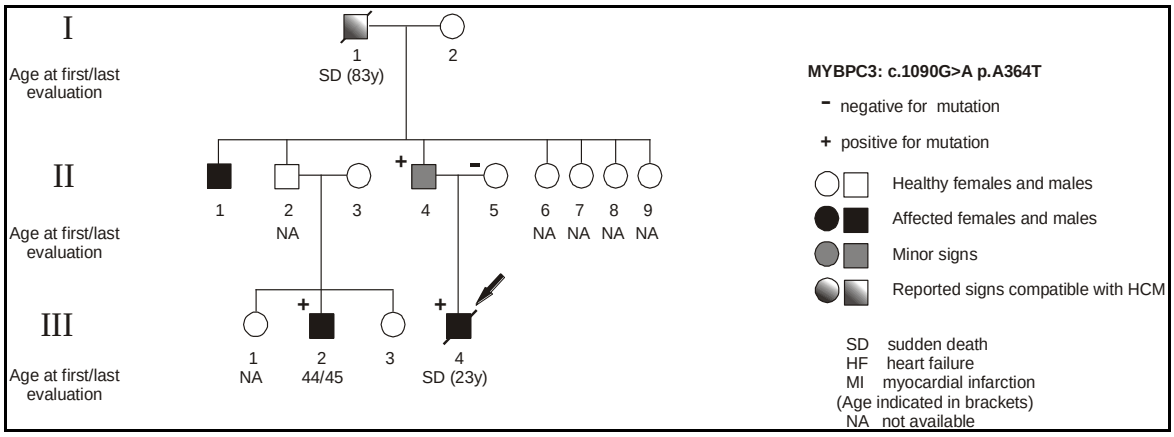

Figure 14. Pedigree of family FA.

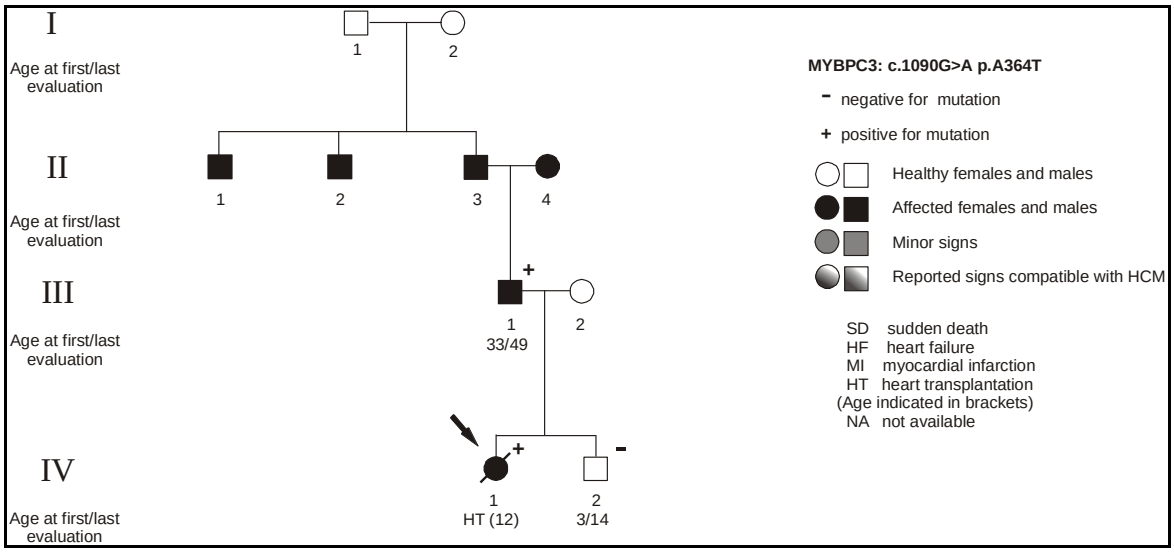

Figure 15. Pedigree of family IU.

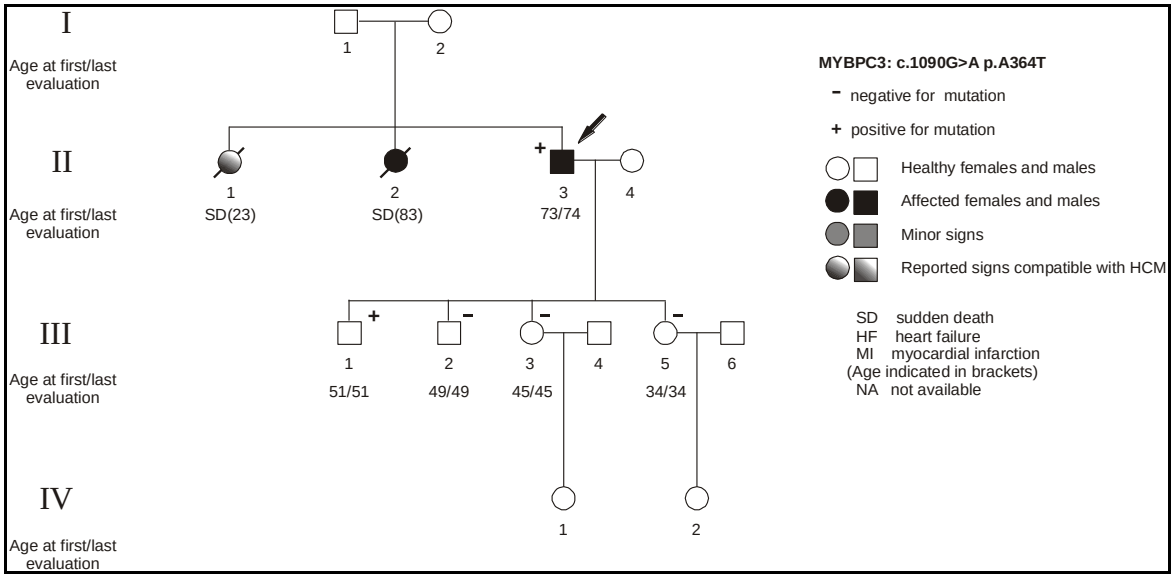

Figure 16. Pedigree of family ME.

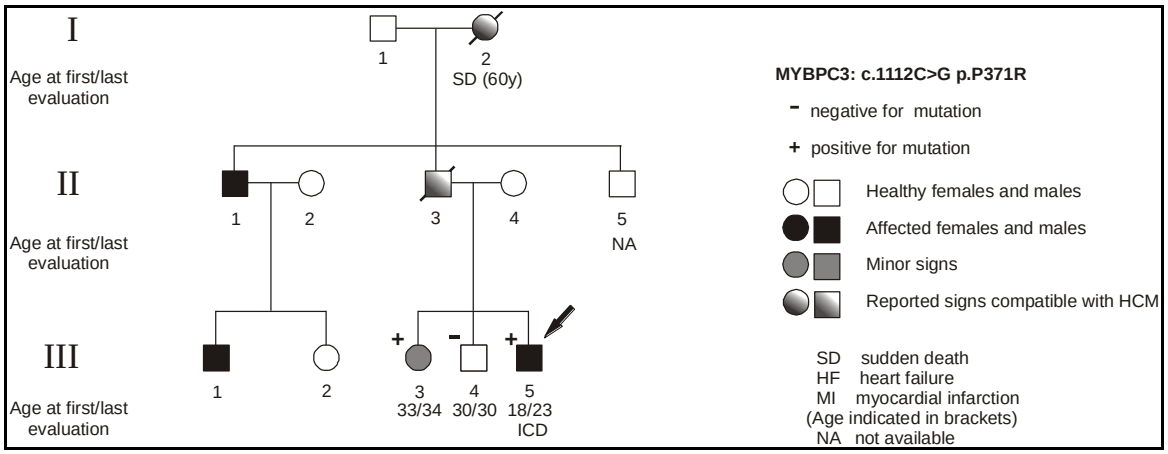

Figure 17. Pedigree of family MO.

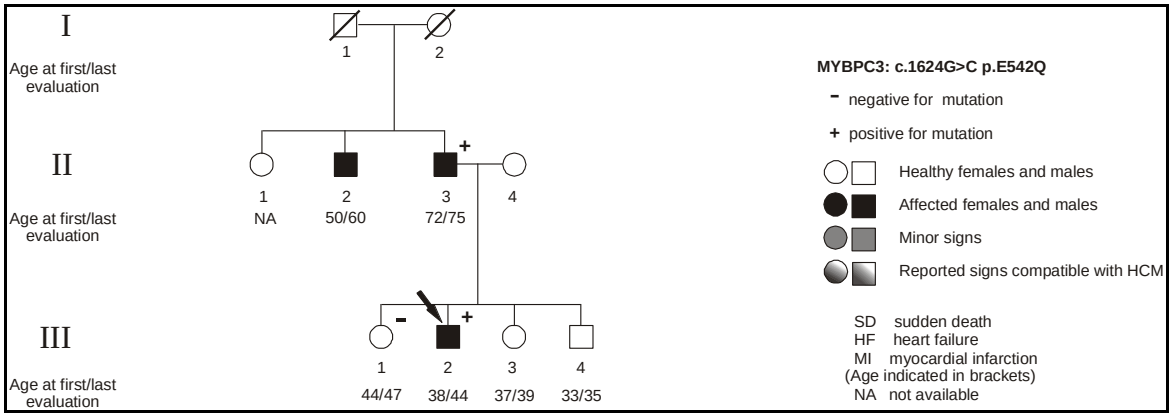

Figure 18. Pedigree of family SM.

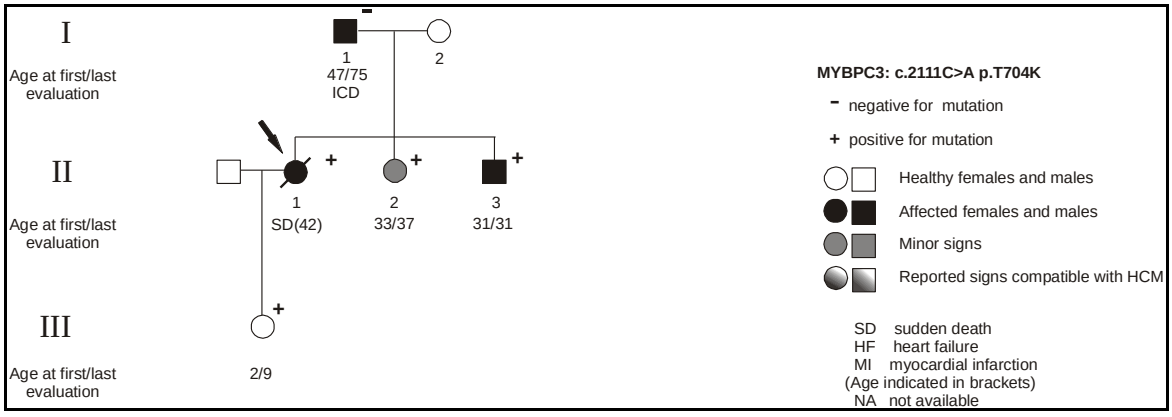

Figure 19. Pedigree of family SC.

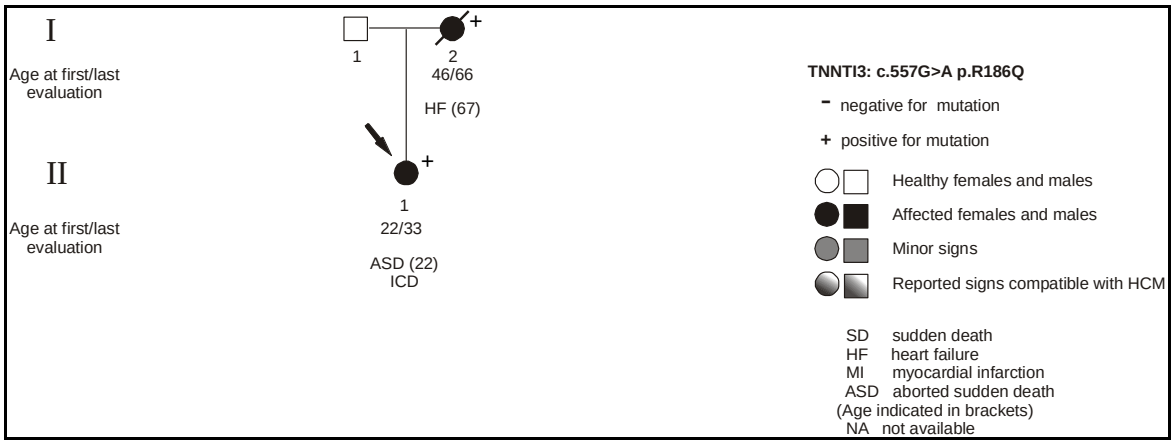

Figure 20. Pedigree of family RI.

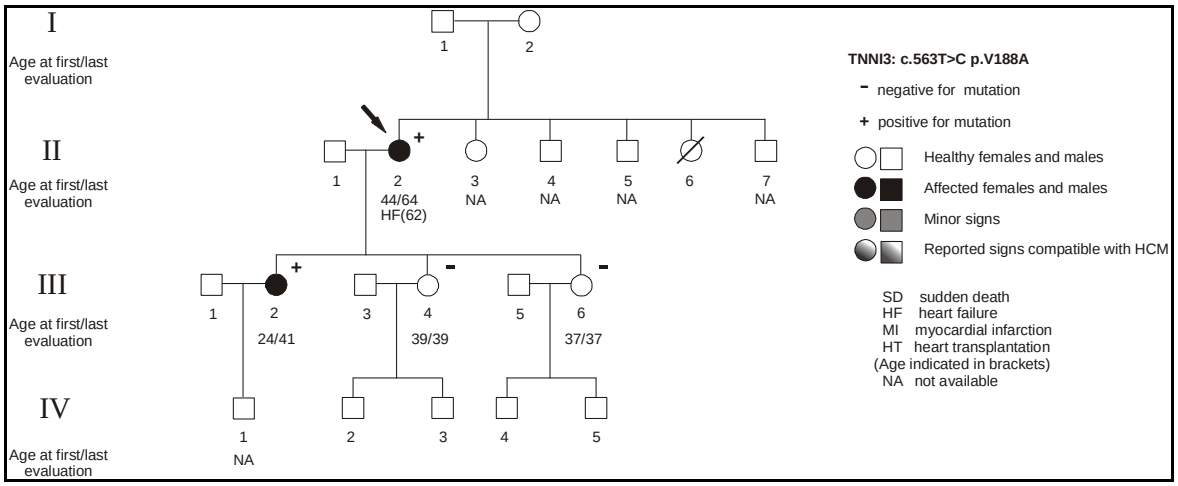

Figure 21. Pedigree of family MG.

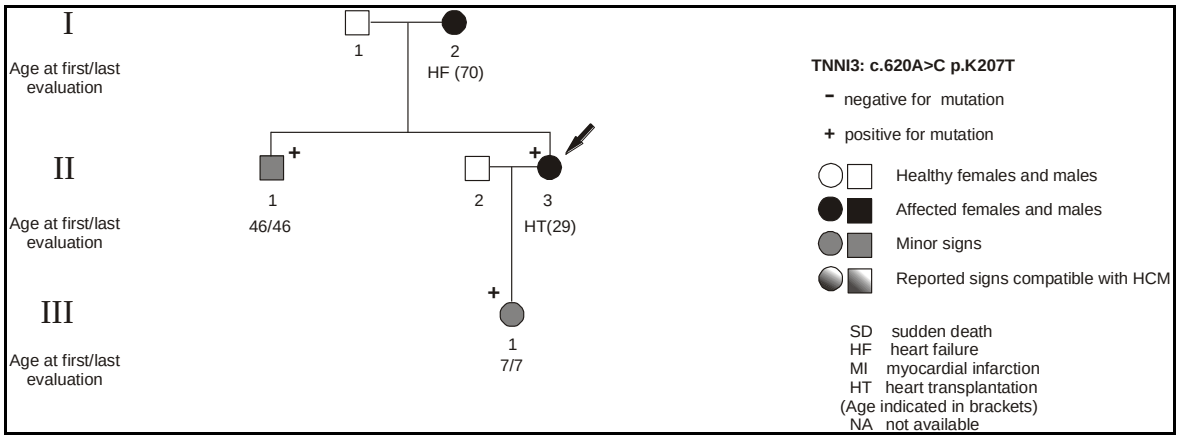

Figure 22. Pedigree of family RA.

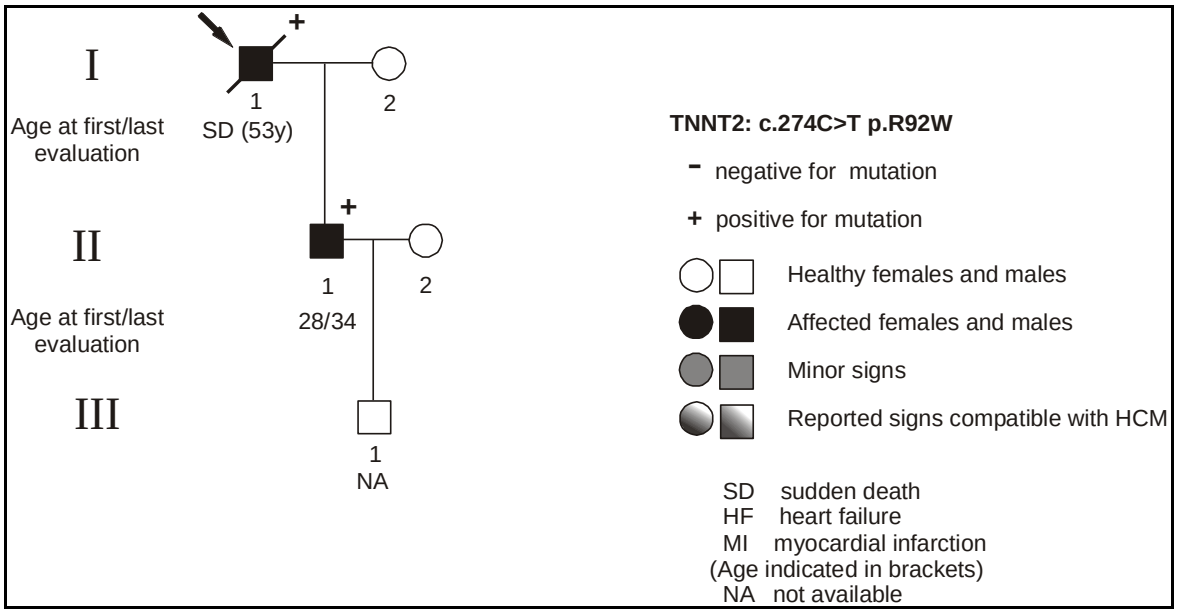

Figure 23. Pedigree of family LO.

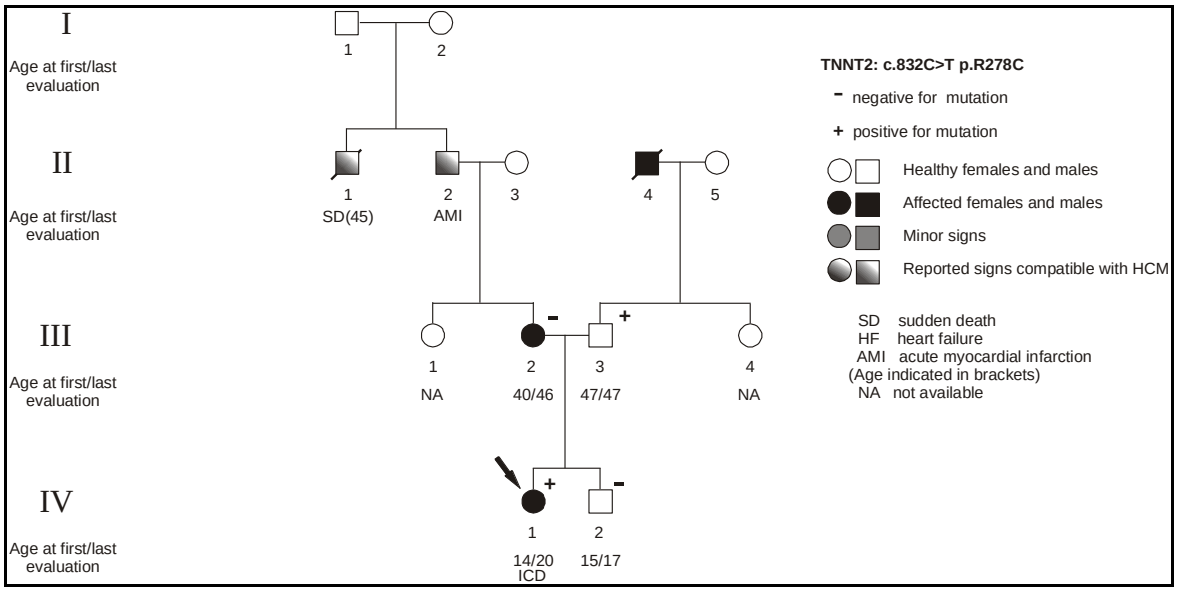

Figure 24. Pedigree of family PF.

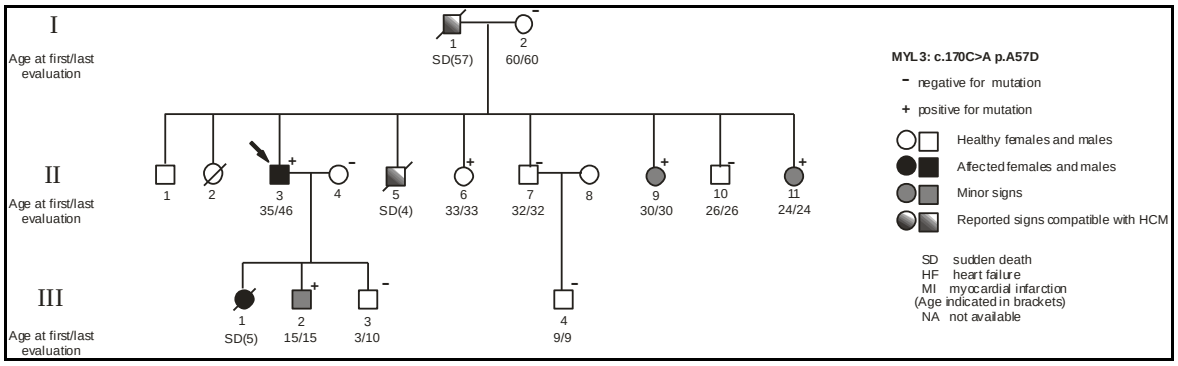

Figure 25. Pedigree of family CA.

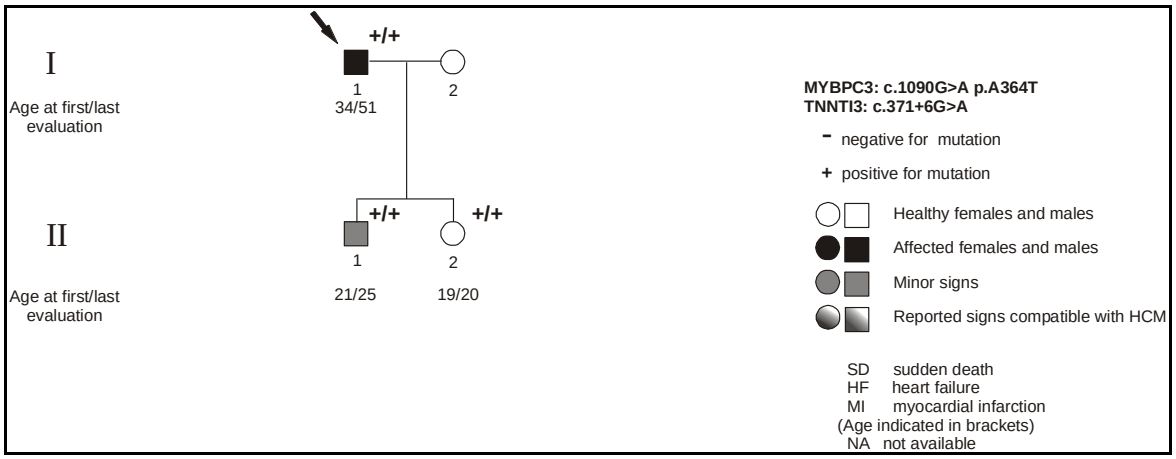

Figure 26. Pedigree of family FE.

Table 17. Characteristics of mutation carriers.

	Total carriers (n=23)	**HCM+ carriers (n=8)**	**Carriers with minor signs of HCM (n=10)**	**Healthy carriers (n=5)**	**p HCM vs minor signs**	**p HCM vs healthy**	**p healthy vs minor signs**
Male, n(%)	12 (52)	6 (75)	4 (40)	2 (40)	0.14	0.2	1
Female, n(%)	11 (48)	2 (25)	6 (60)	3 (60)	0.14	0.2	1
Age at last control, years	38±19	52±17	30±18	34±15	**0.02**	0.08	0.65
MLVWT, mm							
range	6-30	13-30	6-12	6-11			
mean	14±6	19±6	11±2	9±2	**0.003**	**0.005**	0.3
Mass/volume	1.3±0.3	1.6±0.3	1.1±0.2	1.2±0.1	**0.007**	**0.02**	0.8
EF, (%)	59±7	57±8	62±6	57±4	0.26	0.96	0.17
Mitral prolapse, n (%)	12 (52)	4 (50)	5 (50)	3 (60)	1	0.7	0.7
Abnormal LV filling, n (%)	8 (35)	6 (75)	2 (20)	0	**0.02**	**0.008**	0.28
TDI[§]							
Ea vel (cm/sec)	13.4±4.8	9.1±1.3	16.0±3.4	16.7±5.1	**0.013**	**0.034**	0.85
E/Ea	6.0±2.3	8.0±2.4	5.2±0.7	4.2±0.5	0.12	**0.05**	0.1
Asymptomatic pts,n(%)	15 (65)	3 (37)	8 (80)	5 (100)	0.07	**0.02**	0.28
NYHA class, n(%)							
I	20 (87)	5 (62.5)	10 (100)	5 (100)	0.03	0.11	n/a
II	2 (9)	2 (25)	0	0	0.09	0.22	n/a
III	0	0	0	0	n/a	n/a	n/a
IV	1 (4)	1 (12.5)	0	0	0.24	0.41	n/a
Syncope, n (%)	2 (9)	2 (25)	0	0	0.15	0.21	n/a
Abnormal ECG, n (%)	14 (61)	8 (100)	6 (60)	0	0.2	**0.005**	**0.09**
AF, n (%)	0	0	0	0	n/a	n/a	n/a
NSVT, n (%)	2 (10)	2 (25)	0	0	0.09	0.22	n/a
Hypertension, n(%)	2 (10)	2 (22)	0	0	0.09	0.22	n/a
Cardiac events, n(%)	1* (4)	1*(12.5)	0	0	0.24	0.41	n/a

Clinic characteristics of mutation carriers with phenotypic expression of HCM (HCM+) are compared with those of carriers with only minor signs of HCM and with those of healthy carriers. AF, atrial fibrillation; EF, ejection fraction; MLVWT, maximal left ventricular wall thickness; n/a, not applicable; NSVT, non sustained ventricular tachycardia. [§] available in 10 patients; *=heart failure death

Table 18. Clinical characteristics of mutation positive (+) vs mutation negative (-) healthy relatives.

	Total healthy relatives (n=24)	**Healthy relatives Mutation + (n=5)**	**Healthy relatives Mutation - (n=19)**	**P (mut+ vs mut-)**
Male, n (%)	13 (54)	2 (40)	11 (58)	0.47
Female, n (%)	12 (46)	3 (60)	8 (42)	0.47
Age at last control, years				
range	9-60	19-47	9-60	
mean	35 ± 16	34 ± 15	35 ± 17	0.9
MLVWT, mm				
range	5-12	8-11	5-12	
mean	9 ± 2	9 ± 2	9 ± 2	0.67
Mass/volume	1.1 ± 0.1	1.2 ± 0.1	1.0 ± 0.1	0.06
EF, (%)	61 ± 7	57 ± 4	62 ± 7	0.18
Mitral prolapse, n (%)	9 (37)	3 (60)	6 (31)	0.24
Abnormal LV filling, n (%)	3 (12.5)	0	3 (16)	0.34
TDI, n pts available (%)	9 (37)	3 (60)	6 (30)	
Ea wave vel (cm/sec)	15.4 ± 3.9	16.7 ± 5.1	14.7 ± 3.5	0.5
E/Ea	5.3 ± 1.5	4.2 ± 0.5	5.9 ± 1.5	0.1
Asymptomatic pts, n (%)	21 (88)	5 (100)	16 (84)	0.34
NYHA class, n (%)				
I	24 (100)	5 (100)	19 (100)	n/a
II	0	0	0	n/a
III	0	0	0	n/a
IV	0	0	0	n/a
Syncope, n (%)	1 (4)	0	1 (5)	0.6
Abnormal ECG, n (%)	3 (12)	0	3 (16)	0.34
AF, n (%)	0	0	0	1
NSVT, n (%)	0	0	0	1
Hypertension, n (%)	2 (8)	0	2 (10)	0.44
Cardiac events, n (%)	0	0	0	1

AF, atrial fibrillation; EF, ejection fraction; MLVWT, maximal left ventricular wall thickness; n/a, not applicable; NSVT, non sustained ventricular tachycardia.

DISCUSSION

Hypertrophic cardiomyopathy is a complex disease with a broad clinical heterogeneity matched by an equally high degree of genetic complexity.

Definition of HCM as "disease of the sarcomere" is proper since mutations of each components of this macromolecular structure (thick filament, thin filament and associated proteins) can play a pathogenic role in disease development, but the limited percentage of probands harbouring sarcomeric mutation (from 30 to 65% in various cohort studies) should drive our attention beyond the sarcomere for identifying other disease-causing genes.

Our findings are consistent with previous published data, showing a prevalence of sarcomeric mutations of about 30%, not different in probands with HCM family history versus sporadic cases, and irrespective of the screening method used (DHPLC and DNA resequencing array). This percentage is lower than that of about 60% reported by Richard et al (180) and Olivotto et al. (237), but in those studies mutations screening involved 9 and 8 sarcomeric genes respectively, whereas only one-third of our index cases population was screened for 12 genes, most of them been investigated just for the 4 major sarcomeric genes.

Most frequent disease-associated genes were *MYH7* and *MYBPC3* with 11 of 27 (41%) mutations found (10 by DHPLC and one by DNA array) in 11 probands for *MYH7* and 8 of 27 (30%) mutations found (6 by DHPLC and 2 by DNA array) in 12 probands for *MYBPC3*. Frequency of mutations in these 2 genes is lower than that of the abovementioned studies, probably due to different population sampling and/or founder effects. For instance, in Olivotto et al study (237) 27 index cases shared the same *MYBPC3* mutation. Moreover some variants that we have classified as uncommon SNPs (i.e. Ser236Gly in exon 6 of *MYBPC3* shown in Table 16) in other studies have been considered as pathogenic mutations (169).

In the subgroup of 14 index cases analyzed both by DHPLC for 4 genes and DNA array for 12 genes, there was agreement between methods, and only one intronic mutation found by DHPLC was missed by DNA array, thus confirming the low prevalence of insertion-deletions (not detectable by DNA resequencing method) as underlying mechanisms of HCM genetic defect. Therefore, DNA resequencing array resulted an appropriate genetic screening method for HCM in clinical practice with considerable gain in time, labor and cost. On the other hand, DNA array screening for 12 genes

revealed only one pathogenic mutation (in *MYL3* gene) outside the 4 major sarcomeric genes (*MYH7*, *MYBPC3*, *TNNT2* and *TNNI3)*, suggesting to start or focus screening on these before going along with more unusual HCM-causing genes.

Our data, accordingly to current opinions on HCM genetics (106,121,237), did not allow specific genotype-phenotype correlations and showed that also mutations in genes usually associated with benign and late onset HCM forms, as *MYBPC3* (15,179), can lead to severe and malignant phenotypes also in young age (i.e. case # 37, described in Table 9 and Figure 8), as well as mutations in former considered "malignant" genes, as *MYH7*, can correspond to a mild and benign phenotype.

Furthermore, most of mutations found are 'private' (i.e. as yet reported in only one family) and in some cases (i.e. pedigree of family TZ shown in Figure 13) with low penetrance, thereby limiting the prognostic weight that can be attributed to individual mutations.

Anyway, some previously reported (44,116) phenotype-genotype associations were confirmed also in our study population, for example mutations in cardiac troponins I and T genes are often (but not always) associated with mild hypertrophy, restrictive LV filling pattern and adverse prognosis (see Table 10 and Figure 9).

In agreement with Olivotto et al (237), our data seem to indicate that patients harbouring sarcomeric genes mutations had a worse prognosis compared with mutation negative patients, as shown in Kaplan-Meier survival curves in Figure 10.

Patients harbouring multiple mutations are widely recognized as having the most severe disease expression (239) and also our experience are consistent with this. In fact one of our two patients with double mutations (#12 in Table 7) had a particular severe clinical course with sudden death in young age.

Since it is now generally conceded that the identification of specific mutations has little value in predicting prognosis in HCM patients, the primary role for such testing remains the diagnosis of relatives in families with documented HCM, or possibly in probands for whom definitive diagnosis is ambiguous by standard clinical strategies, e.g. in distinguishing HCM from physiological 'athlete's heart' (240).

Indeed, there is substantial justification for pursuing and encouraging the genetic screening of families for HCM in order to recognize affected individuals who would otherwise be unaware of their disease. When a mutation is successfully identified in a proband, accurate definition of genetic status can be achieved in all family members much more efficiently and inexpensively. Recent studies (107) have demonstrated that

gene-based diagnostic strategies in families have economic as well as medical benefits. Genetic testing results in particular if negative, will reduce the number of family members who require serial follow-up, leading to healthcare cost-saving.

In our study cohort of relatives, about one-half (45%) resulted positive for their family proband mutation and this is consistent with an autosomal dominant inheritance. Eight (35%) relatives had phenotypic expression of the disease, whereas the remaining carriers had no (22%) or only minor signs (43%) of HCM, and, as shown in Table 17, HCM affected carriers were older than phenotype uncertain or healthy carriers thus confirming the incomplete and age-related penetrance of the disease, and suggesting that cardiological evaluations should continue until advanced age. Carriers with clinical-morphologic signs suggestive of HCM, but not diagnostic, as ECG abnormalities, mitral prolapse, LV hypertrophy with maximal LVWT of 12-13 mm, deserve particular attention, because they probably may show initial expression of the disease and should be carefully followed with high sensitive diagnostic exam as 3D echo with strain and CMR. Interestingly, our data confirmed that TDI imaging measurements, as recently reported by Gandjbakhch et al (79), are associated with genetic status, but TDI velocities alone were not reliable enough to identify LVH-free mutation carriers, so multiparametric scores, combining electrocardiographic, echocardiographic and TDI parameters, are needed to identify mutation carriers before and independently of hypertrophy with high accuracy.

While family members who have not inherited a pathogenic mutation can be reassured that neither they nor their offspring are at risk of disease, the natural history, risk of sudden death, general prognosis, potential management strategies and decision making related to mutation carriers is not only currently unresolved, but also essentially unknown given the youthful age of the affected relatives and the limited follow-up available in this subset, which requires substantial periods of longitudinal observation. Also unknown is which (and how commonly) gene+/phenotype- individuals will ultimately develop the HCM phenotype, or at what time in life (240). Such uncertainty may impact on the question of eligibility vs disqualification from intense competitive sports, as well as the wisdom of prophylactic implantation of cardioverter-defibrillators, particularly if there is a family history of HCM-related sudden death. For example, only very recently has the first report appeared in the literature of two gene carriers incurring sudden death events, offering support for the principle that the non-hypertrophied LV muscle in these individuals can represent an arrhythmogenic substrate (241). On the

opposite, the same Dutch group have recently published a large HCM carriers population study showing that gene+/phenotype- patients had a low risk for SD (122).
Up to date, the expanding preclinical genotype+/phenotype- HCM subgroup represents a major challenge to clinicians. Answers to the prevailing important questions concerning management decisions for such individuals will unavoidably require many years of careful assembly of data in large cohorts with longitudinal and substantial follow-up. On the other hand, identification of early phenotypes, characterized by structural abnormalities (as fibrosis, impaired myocardial energetic, diastolic dysfunction) in absence of LV hypertrophy, may highlight previously unrecognized pathway that contribute to disease development and may be targeted therapeutically changing the natural history of this disease (242) .

Limitations of the study

Owing to the high cost of the genetic screening (completely funded by research grants) it was not feasible to submit consecutively all patients to DNA analysis, so we decided to select index cases with particularly severe HCM phenotype or belonging to large and/or "malignant" HCM families, characterized by high incidence of sudden or heart-failure related deaths and cardiac transplants, and to proceed with a "stepwise" genetic testing as reported in the flow-chart in Figure 5.
Moreover, only one-third of our index-cases population has been screened for 12 genes, whereas most of them have been investigated just for the 4 major sarcomeric genes.
Kaplan-Meier survival curves in Figure 10 showed a worse prognosis in patients harbouring sarcomeric genes mutations compared with mutation negative patients, but caution is needed in reading these data for several reasons. First of all, we must be aware of heterogeneity inside each group: mutation negative probands could represent phenocopies, as well as probands with mutation in yet-to-be-identified HCM-susceptibility genes or novel myofilament-encoding genes or unexplored regions of sarcomeric genes. Furthermore, our study population index-cases were selected from "malignant" families with high rate of unfavourable outcome and they do not represent general outpatients HCM population.

CONCLUSIONS

Mutation screening is becoming part of diagnostic and clinical management of HCM patients and family members.

In spite of genotype characterization, a wide heterogeneity in clinical presentation and evolution is present, but when multiple mutations are detected, they are constantly associated with particularly severe phenotype.

Since the low percentage of sarcomeric mutations identified in probands, genetic research is moving toward new genes, in particular Z-disk genes, calcium-handling, and gene associated with metabolic/storage cardiomyopathies. Moreover, advance in technology will allow a more comprehensive, cost-effective and time-saving genetic screening.

Efforts to refine and clinically implement genetic testing in HCM could also serve as a model for other genetic cardiovascular diseases. By identifying at-risk individuals prior to clinical diagnosis, characterizing disease pathogenesis, and fostering development of novel therapies to delay or prevent phenotypic expression, we believe that genetic discoveries will improve the lives of our patients with HCM.

REFERENCES

1. Liouville H. Retrecissement cardiaque sous aortique. *Gaz Med Paris.* 1869; 24: 161.

2. Hallopeau L. Retrecissement ventriculo-aortique. *Gaz Med Paris.* 1869; 24: 683.

3. Brock R. Functional obstruction of the left ventricle. *Guy's Hospital Rep.* 1957; 106:221.

4. Teare D. Asymmetrical hypertrophy of the heart in young adults. *Br Heart J.* 1958; 20: 1–8.

5. Frank S, Braunwald E. Idiopathic hypertrophic subaortic stenosis: clinical analysis of 126 patients with emphasis on the natural history. *Circulation.* 1968; 37: 759–88.

6. Goodwin JF, Hollman A, Cleland WP, Teare D. Obstructive cardiomyopathy simulating aortic stenosis. *Br Heart J.* 1960; 22: 403–14.

7. Wigle ED, Heimbecker RO, Gunton RW. Idiopathic ventricular septal hypertrophy causing muscular subaortic stenosis. *Circulation.* 1962; 26: 325–40.

8. Shah PM, Gramiak R, Kramer DH. Ultrasound localization of left ventricular outflow obstruction in hypertrophic obstructive cardiomyopathy. *Circulation.* 1969; 40: 3–11.

9. Maron BJ, Gottdiener JS, Epstein SE. Patterns and significance of the distribution of left ventricular hypertrophy in hypertrophic cardiomyopathy: a wide angle, two-dimensional echocardiographic study of 125 patients. *Am J Cardiol.* 1981; 48: 418–28.

10. Klues HG, Schiffers A, Maron BJ. 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;26:1699–708.

11. Maron BJ, McKenna WJ, Danielson GK, Kappenberger LJ, Kuhn HJ, Seidman CE, Shah PM, Spencer WH, Spirito P, Ten Cate FJ, Wigle ED. ACC/ESC clinical expert consensus document on hypertrophic cardiomyopathy: a report of the American College of Cardiology Task Force on Clinical Expert Consensus Documents and the European Society of Cardiology Committee for Practice Guidelines (Committee to Develop an Expert Consensus Document on Hypertrophic Cardiomyopathy). *J Am Coll Cardiol.* 2003;42:1687–713.

12. Maron BJ, Moller JH, Seidman CE, Vincent GM, Dietz HC, Moss AJ, Towbin JA, Sondheimer HM, Pyeritz RE, McGee G, Epstein AE. Impact of laboratory molecular diagnosis on contemporary diagnostic criteria for genetically transmitted cardiovascular diseases: hypertrophic cardiomyopathy, long-QT syndrome, and Marfan syndrome. [A statement for healthcare professionals from the Councils on Clinical Cardiology, Cardiovascular Disease in the Young, and Basic Science, American Heart Association]. *Circulation.* 1998;98:1460–71.

13. Seidman JG, Seidman CE. The genetic basis for cardiomyopathy: from mutation identification to mechanistic paradigms. *Cell.* 2001;104:557–67.

14. Charron P, Dubourg O, Desnos M, Bennaceur M, Carrier L, Camproux AC, Isnard R, Hagege A, Langlard JM, Bonne G, Richard P, Hainque B, Bouhour JB, Schwartz K, Komajda M. Clinical features and prognostic implications of familial hypertrophic cardiomyopathy related to the cardiac myosin-binding protein C gene. *Circulation.* 1998;97:2230–6.

15. Maron BJ, Niimura H, Casey SA, Soper MK, Wright GB, Seidman JG, Seidman CE. Development of left ventricular hypertrophy in adults in hypertrophic cardiomyopathy caused by cardiac myosin-binding protein C gene mutations. *J Am Coll Cardiol.* 2001;38:315–21.

16. McKenna WJ, Spirito P, Desnos M, Dubourg O, Komajda M. Experience from clinical genetics in hypertrophic cardiomyopathy: proposal for new diagnostic criteria in adult members of affected families. *Heart.* 1997;77:130–2.

17. Hada Y, Sakamoto T, Amano K, Yamaguchi T, Takenaka K, Takahashi H, Takikawa R, Hasegawa I, Takahashi T, Suzuki J. Prevalence of hypertrophic cardiomyopathy in a population of adult Japanese workers as detected by echocardiographic screening. *Am J Cardiol.* 1987; 59:183–84.

18. Maron BJ, Gardin JM, Flack JM, Gidding SS, Kurosaki TT, Bild DE. Prevalence of hypertrophic cardiomyopathy in a population of young adults. Echocardiographic analysis of 4111 subjects in the CARDIA study. Coronary Artery Risk Development in (Young) Adults. *Circulation.* 1995; 92: 785–89.

19. Codd MB, Sugrue DD, Gersh BJ, Melton LJ. Epidemiology of idiopathic dilated and hypertrophic cardiomyopathy: a population based study in Olmsted County, Minnesota, 1975–1984. *Circulation.* 1989; 80: 564–72.

20. Maron BJ, Peterson EE, Maron MS, Peterson JE. Prevalence of hypertrophic cardiomyopathy in an outpatient population referred for echocardiographic study. *Am J Cardiol.* 1994; 73: 577–80.

21. Lipshultz SE, Sleeper LA, Towbin JA, Lowe AM, Orav EJ, Cox GF, Lurie PR, McCoy KL, McDonald MA, Messere JE, Colan SD. The incidence of pediatric cardiomyopathy in two regions of the United States. *N Engl J Med.* 2003; 348: 1647–55.

22. Nugent AW, Daubeney PE, Chondros P, Carlin JB, Cheung M, Wilkinson LC, Davis AM, Kahler SG, Chow CW, Wilkinson JL, Weintraub RG; National Australian Childhood Cardiomyopathy Study. The Epidemiology of Childhood Cardiomyopathy in Australia. *N Engl J Med.* 2003; 348**:**1639–46.

23. Webb JG, Sasson Z, Rakowski H, Liu P, Wigle ED. Apical hypertrophic cardiomyopathy. *J Am Coll Cardiol.* 1990;15:83-90.

24. Obeid AI, Maron BJ. Apical hypertrophic cardiomyopathy developing at a relatively advance age. *Circulation.* 2001;103:1605.

25. Yamaguchi H, Ishimura T, Nishiyama S, Nagasaki F, Nakanishi S, Takatsu F, Nishijo T, Umeda T, Machii K. Hypertrophic nonobstructive cardiomyopathy with giant negative T waves (apical hypertrophy): ventriculographic and echocardiographic features in 30 patients. *Am J Cardiol.* 1979;44:401–12.

26. Maron BJ, Spirito P, Green KJ, Wesley YE, Bonow RO, Arce J. Noninvasive assessment of left ventricular diastolic function by pulsed Doppler echocardiography in patients with hypertrophic cardiomyopathy. *J Am Coll Cardiol.* 1987;10:733–42.

27. Wigle ED. Cardiomyopathy: The diagnosis of hypertrophic cardiomyopathy. *Heart.* 2001;86:709–14.

28. Grigg LE, Wigle ED, Williams WG, Daniel LB, Rakowski H. Transesophageal Doppler echocardiography in obstructive hypertrophic cardiomyopathy: clarification of pathophysiology and importance in intraoperative decision making. *J Am Coll Cardiol.* 1992;20:42–52.

29. Klues HG, Roberts WC, Maron BJ. Anomalous insertion of papillary muscle directly into anterior mitral leaflet in hypertrophic cardiomyopathy. Significance in producing left ventricular outflow obstruction. *Circulation.* 1991;84:1188–97.

30. Petrone RK, Klues HG, Panza JA, Peterson EE, Maron BJ. Coexistence of mitral valve prolapse in a consecutive group of 528 patients with hypertrophic cardiomyopathy assessed with echocardiography. *J Am Coll Cardiol.* 1992;20:55–61.

31. Maron BJ, Sato N, Roberts WC, Edwards JE, Chandra RS. Quantitative analysis of cardiac muscle cell disorganisation in the ventricular septum: comparison of fetuses and infants with and without congenital heart disease and patients with hypertrophic cardiomyopathy. *Circulation.* 1979; 60: 685–96.

32. Factor SM, Butany J, Sole MJ, Wigle ED, Williams WC, Rojkind M. Pathological fibrosis and matrix connective tissue in the subaortic myocardium of patients with hypertrophic cardiomyopathy. *J Am Coll Cardiol.* 1991; 17**:** 1343–51.

33. Shirani J, Pick R, Roberts WC, Maron BJ. Morphology and significance of the left ventricular collagen network in young patients with hypertrophic cardiomyopathy and sudden cardiac death. *J Am Coll Cardiol.* 2000; 35: 36–44.

34. Varnava AM, Elliott PM, Sharma S, McKenna WJ, Davies MJ. Hypertrophic cardiomyopathy: the interrelation of disarray, fibrosis, and small vessel disease. *Heart.* 2000; 84: 476–82.

35. Maron BJ, Wolfson JK, Epstein SE, Roberts WC. Intramural ("small vessel") coronary artery disease in hypertrophic cardiomyopathy. *J Am Coll Cardiol*. 1986; 8: 545–57.

36. Tanaka M, Fujiwara H, Onodera T, Wu DJ, Matsuda M, Hamashima Y, Kawai C Quantitative analysis of narrowings of intramyocardial small arteries in normal hearts, hypertensive hearts, and hearts with hypertrophic cardiomyopathy. *Circulation.* 1987; 75: 1130–39.

37. Cannon RO, Rosing DR, Maron BJ, Leon MB, Bonow RO, Watson RM, Epstein SE. Myocardial ischemia in patients with hypertrophic cardiomyopathy: contribution of inadequate vasodilator reserve and elevated left ventricular filling pressures. *Circulation.* 1985; 71: 234–237.

38. Elliott PM, Rosano GMC, Gill JS, Poole-Wilson PA, Kaski JC, McKenna WJ. Changes in coronary sinus pH during dipyridamole stress in patients with hypertrophic cardiomyopathy. *Heart.* 1996; 75: 179–83.

39. Krams R. Decreased coronary flow riserve in hypertrophic cardiomyopathy is related to remodelling of the coronary microcirculation. *Circulation.* 1998; 97:230-233.

40. Basso C, Thiene G, Corrado D, Buja G, Melacini P, Nava A. Hypertrophic cardiomyopathy and sudden death in the young: pathologic evidence of myocardial ischemia. *Hum Pathol*. 2000;31:988-998.

41. Gori F, Basso C, Thiene G. Myocardial infarction in a patient with hypertrophic cardiomyopathy. *N Engl J Med*. 2000;342:593-4.

42. Basso C, Thiene G, Mackey-Bojack S, Frigo AC, Corrado D, Maron BJ. Myocardial bridging, a frequent component of the hypertrophic cardiomyopathy phenotype, lacks systematic association with sudden cardiac death. *Eur Heart J*. 2009;30:1627-34.

43. Nicod P, Polikar R, Peterson KL. Hypertrophic cardiomyopathy and sudden death. *N Engl J Med.* 1998;318:1255-1257.

44. Mogensen J, Kubo T, Duque M, Uribe W, Shaw A, Murphy R, Gimeno JR, Elliott P, McKenna WJ. Idiopathic restrictive cardiomyopathy is part of the clinical expression of cardiac Troponin I mutations. *J Clin Invest.* 2003; 111: 209–16.

45. Waller BF, Maron BJ, Morrow AG, Roberts WC. Hypertrophic cardiomyopathy mimicking pericardial constriction or myocardial restriction. *Am Heart J.* 1981; 102: 790–92.

46. McKenna WJ, Stewart JT, Nihoyannopoulos P, McGinty F, Davies MJ. Hypertrophic cardiomyopathy without hypertrophy: two families with myocardial disarray in the absence of increased myocardial mass. *Br Heart J.* 1990; 63: 287–90.

47. Ohsato K, Shimizu M, Sugihara N, Konishi K, Takeda R. Histopathological factors related to diastolic function in myocardial hypertrophy. *Jpn Circ J.* 1992;56:325–333.

48. Pak PH, Maughan L, Baughman KL, Kass DA. Marked discordance between dynamic and passive diastolic pressure-volume relations in idiopathic hypertrophic cardiomyopathy. *Circulation*. 1996;94:52– 60.

49. Rakowski H and Carasso S. Quantifying Diastolic Function in Hypertrophic Cardiomyopathy: The Ongoing Search for the Holy Grail. *Circulation.* 2007;116:2662-2665.

50. Maron BJ, Epstein SE, Roberts WC. Hypertrophic cardiomyopathy and transmural myocardial infarction without significant atherosclerosis of the extramural coronary arteries. *Am J Cardiol.* 1979;43:1086–102.

51. Cecchi F, Olivotto I, Gistri R, Lorenzoni R, Chiriatti G, Camici PG. Coronary microvascular dysfunction and prognosis in hypertrophic cardiomyopathy. *N Engl J Med.* 2003;349:1027-35.

52. Cannon RO, Dilsizian V, O'Gara PT, Udelson JE, Schenke WH, Quyyumi A, Fananapazir L, Bonow RO. Myocardial metabolic, hemodynamic and electrocardiographic significance of reversible thallium-201 abnormalities in hypertrophic cardiomyopathy. *Circulation.* 1991;83:1660-7.

53. Maron BJ, Wolfson JK, Epstein SE, Roberts WC. Intramural (''small vessel'') coronary artery disease in hypertrophic cardiomyopathy. *J Am Coll Cardiol.* 1986;8:545-57.

54. Cannon RO III, Rosing DR, Maron BJ, Leon MB, Bonow RO, Watson RM, Epstein SE. Myocardial ischemia in patients with hypertrophic cardiomyopathy: contribution of inadequate vasodilator reserve and elevated left ventricular filling pressures. *Circulation.* 1985;71:234-43.

55. Melacini P, Corbetti F, Calore C, Pescatore V, Smaniotto G, Pavei A, Bobbo F, Cacciavillani L, Iliceto S. Cardiovascular magnetic resonance signs of ischemia in hypertrophic cardiomyopathy**.** *Intern J Cardiol.* 2008;128:364-373.

56. Kizilbash AM, Heinle SK, Grayburn PA. Spontaneous variability of left ventricular outflow tract gradient in hypertrophic obstructive cardiomyopathy. *Circulation.* 1998;97:461–6.

57. Paz R, Jortner R, Tunick PA, Sclarovsky S, Eilat B, Perez JL, Kronzon I. The effect of the ingestion of ethanol on obstruction of the left ventricular outflow tract in hypertrophic cardiomyopathy. *N Engl J Med.* 1996;335:938–41.

58. Maron MS, Olivotto I, Betocchi S, Casey SA, Lesser JR, Losi MA, Cecchi F, Maron BJ. Effect of left ventricular outflow tract obstruction on clinical outcome in hypertrophic cardiomyopathy. *N Engl J Med.* 2003;348:295–303.

59. Maron BJ. Hypertrophic Cardiomyopathy A systematic review. *JAMA.* 2002;287:1308-1320.

60. Choudhury L, Mahrholdt H, Wagner A, Choi KM, Elliott MD, Klocke FJ, Bonow RO, Judd RM, Kim RJ. Myocardial scarring in asymptomatic or mildly symptomatic patients with hypertrophic cardiomyopathy. *J Am Coll Cardiol.* 2002;40:2156–64.

61. Spirito P, Seidman CE, McKenna WJ, Maron BJ. The management of hypertrophic cardiomyopathy. *N Engl J Med.* 1997;336:775–85.

62. Elliott P, Mc Kenna WJ. Hypertrophic Cardiomyopathy. *Lancet.* 2004;363:1881-91.

63. Maron BJ. The electrocardiogram as a diagnostic tool for hypertrophic cardiomyopathy: revisited. *Ann Noninvasive Electrocardiol*. 2001;6:277-279.

64. Monserrat L, Elliott PM, Gimeno JR, Sharma S, Penas-Lado M, McKenna WJ. Non-sustained ventricular tachycardia in hypertrophic cardiomyopathy: an independent marker of sudden death risk in young patients. *J Am Coll Cardiol.* 2003; 42: 873–79.

65. Maron BJ, Savage DD, Wolfson JK, Epstein SE. Prognostic significance of 24 hour ambulatory electrocardiographic monitoring in patients with hypertrophic cardiomyopathy: a prospective study. *Am J Cardiol.* 1981; 48: 252–57.

66. Robinson K, Frenneaux MP, Stockins B, Karatasakis G, Poloniecki J, McKenna WJ. Atrial fibrillation in hypertrophic cardiomyopathy: A longitudinal study. *J Am Coll Cardiol.* 1990; 15: 1279–85.

67. Olivotto I, Cecchi F, Casey SA, Dolara A, Traverse JH, Maron BJ. Impact of atrial fibrillation on the clinical course of hypertrophic cardiomyopathy. *Circulation.* 2001; 104: 2517–24.

68. Maron BJ, Olivotto I, Bellone P, Conte MR, Cecchi F, Flygenring BP, Casey SA, Gohman TE, Bongioanni S, Spirito P. Clinical profile of stroke in 900 patients with hypertrophic cardiomyopathy. *J Am Coll Cardiol.* 2002; 39: 301–07.

69. Alfonso F, Frenneaux MP, McKenna WJ. Clinical sustained uniform ventricular tachycardia in hypertrophic cardiomyopathy: association with left ventricular apical aneurysm. *Br Heart J.* 1989; 61: 178–81.

70. Nagueh SF, Bierig SM, Budoff MJ, Desai M, Dilsizian V, Eidem B, Goldstein SA, Hung J, Maron MS, Ommen SR, Woo A; American Society of Echocardiography; American Society of Nuclear Cardiology; Society for Cardiovascular Magnetic Resonance; Society of Cardiovascular Computed Tomography. American Society of

Echocardiography Clinical Recommendations for Multimodality Cardiovascular Imaging of Patients with Hypertrophic Cardiomyopathy. *J Am Soc Echocardiogr*. 2011;24:473-98.

71. Olszewski R, Timperley J, Szmigielski C, Monaghan M, Nihoyannopoulos P, Senior R, Becher H. The clinical applications of contrast echocardiography. *Eur J Echocardiogr*. 2007;8:S13-23.

72. Mulvagh SL, Rakowski H, Vannan MA, Abdelmoneim SS, Becher H, Bierig SM, Burns PN, Castello R, Coon PD, Hagen ME, Jollis JG, Kimball TR, Kitzman DW, Kronzon I, Labovitz AJ, Lang RM, Mathew J, Moir WS, Nagueh SF, Pearlman AS, Perez JE, Porter TR, Rosenbloom J, Strachan GM, Thanigaraj S, Wei K, Woo A, Yu EH, Zoghbi WA. American Society of Echocardiography consensus statement on the clinical applications of ultrasonic contrast agents in echocardiography. *J Am Soc Echocardiogr*. 2008;21:1179-201.

73. Spirito P, Maron BJ. Relation between extent of left ventricular hypertrophy and occurrence of sudden cardiac death in hypertrophic cardiomyopathy. *J Am Coll Cardiol.* 1990;15:1521-6.

74. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise JS, Solomon SD, Spencer KT, Sutton MS, Stewart WJ. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. *J Am Soc Echocardiogr*. 2005;18:1440-63.

75. Caselli S, Pelliccia A, Maron M, Santini D, Puccio D, Marcantonio A, Pandian NG, De Castro S. Differentiation of hypertrophic cardiomyopathy from other forms of left ventricular hypertrophy by means of three-dimensional echocardiography. *Am J Cardiol.* 2008;102:616-20.

76. Thaman R, Gimeno JR, Murphy RT, Kubo T, Sachdev B, Mogensen J, Elliott PM, McKenna WJ. Prevalence and clinical significance of systolic impairment in hypertrophic cardiomyopathy. *Heart.* 2005;91:920-5.

77. Nagueh SF, Bachinski LL, Meyer D, Hill R, ZoghbiWA, Tam JW, et al. Tissue Doppler imaging consistently detects myocardial abnormalities in patients with

hypertrophic cardiomyopathy and provides a novel means for an early diagnosis before and independently of hypertrophy. *Circulation.* 2001;104:128-30.

78. Cardim N, Perrot A, Ferreirat T, Pereira A, Osterziel KJ, Reis RP, Correia JF. Usefulness of Doppler myocardial imaging for identification of mutation carriers of familial hypertrophic cardiomyopathy. *Am J Cardiol.* 2002;90:128-32.

79. Gandjbakhch E, Gackowski A, Tezenas du Montcel, S Isnard R, Hamroun A, Richard P, Komajda M, Charron P. Early identification of mutation carriers in familial hypertrophic cardiomyopathy by combined echocardiography and tissue Doppler imaging. *Eur Heart J.* 2010;31:1599-607.

80. Kato TS, Noda A, Izawa H, Yamada A, Obata K, Nagata K, Iwase M, Murohara T, Yokota M. Discrimination of nonobstructive hypertrophic cardiomyopathy from hypertensive left ventricular hypertrophy on the basis of strain rate imaging by tissue Doppler ultrasonography. *Circulation.* 2004;110:3808-14.

81. Serri K, Reant P, Lafitte M, Berhouet M, Le Bouffos V, Roudaut R, Lafitte S. Global and regional myocardial function quantification by twodimensional strain: application in hypertrophic cardiomyopathy. *J Am Coll Cardiol.* 2006;47:1175-81.

82. Carasso S, Yang H, Woo A, Vannan MA, Jamorski M,Wigle ED, Rakowski H. Systolic myocardial mechanics in hypertrophic cardiomyopathy: novel concepts and implications for clinical status. *J Am Soc Echocardiogr.* 2008;21: 675-83.

83. Nishimura RA, Appleton CP, Redfield MM, Ilstrup DM, Holmes DR Jr., Tajik AJ. Noninvasive Doppler echocardiographic evaluation of left ventricular filling pressures in patients with cardiomyopathies: a simultaneous Doppler echocardiographic and cardiac catheterization study. *J Am Coll Cardiol.* 1996;28:1226-33.

84. Nagueh SF, Lakkis NM, Middleton KJ, Spencer WH III, Zoghbi WA, Quinones MA. Doppler estimation of left ventricular filling pressures in patients with hypertrophic cardiomyopathy. *Circulation.* 1999;99:254-61.

85. Matsumura Y, Elliott PM, VirdeeMS, Sorajja P, Doi Y, McKenna WJ. Left ventricular diastolic function assessed using Doppler tissue imaging in patients with hypertrophic cardiomyopathy: relation to symptoms and exercise capacity. *Heart.* 2002;87:247-51.

86. McMahon CJ, Nagueh SF, Pignatelli RH, Denfield SW, Dreyer WJ, Price JF, Clunie S, Bezold LI, Hays AL, Towbin JA, Eidem BW. Characterization of left

ventricular diastolic function by tissue Doppler imaging and clinical status in children with hypertrophic cardiomyopathy. *Circulation.* 2004;109:1756-62.

87. Maron BJ, Nishimura RA, Danielson GK. Pitfalls in clinical recognition and a novel operative approach for hypertrophic cardiomyopathy with severe outflow obstruction due to anomalous papillary muscle. *Circulation.* 1998; 98**:** 2505–08.

88. Cannon RO, Dilsizian V, O'Gara PT, Udelson JE, Tucker E, Panza JA, Fananapazir L, McIntosh CL, Wallace RB, Bonow RO. Impact of operative relief of outflow obstruction on thallium perfusion abnormalities in hypertrophic cardiomyopathy. *Circulation.* 1992;85:1039-45.

89. Camici P, Chiriatti G, Lorenzoni R, Bellina RC, Gistri R, Italiani G, Parodi O, Salvadori PA, Nista N, Papi L. Coronary vasodilation is impaired in both hypertrophied and nonhypertrophied myocardium of patients with hypertrophic cardiomyopathy: a study with nitrogen-13 ammonia and positron emission tomography. *J Am Coll Cardiol.* 1991;17:879-86.

90. Pennel DJ.Cardiovascular magnetic resonance. *Circulation*. 2010;121:692–705.

91. Maron MS, Maron BJ, Harrigan C, Buros J, Gibson CM, Olivotto I, Biller L, Lesser JR, Udelson JE, Manning WJ, Appelbaum E. Hypertrophic cardiomyopathy phenotype revisited after 50 years with cardiovascular magnetic resonance. *J Am Coll Cardiol.* 2009;54:220-8.

92. Rickers C, Wilke NM, Jerosch-Herold M, Casey SA, Panse P, Panse N, Weil J, Zenovich AG, Maron BJ. Utility of cardiac magnetic resonance imaging in the diagnosis of hypertrophic cardiomyopathy. *Circulation.* 2005;112:855-61.

93. Moon JC, Fisher NG, McKenna WJ, Pennell DJ. Detection of apical hypertrophic cardiomyopathy by cardiovascular magnetic resonance in patients with non-diagnostic echocardiography. *Heart.* 2004;90:645-9.

94. Maron MS, Finley JJ, Bos JM, Hauser TH, Manning WJ, Haas TS, Lesser JR, Udelson JE, Ackerman MJ, Maron BJ. Prevalence, clinical significance, and natural history of left ventricular apical aneurysms in hypertrophic cardiomyopathy. *Circulation.* 2008;118:1541-9.

95. Maron MS, Hauser TH, Dubrow E, Horst TA, Kissinger KV, Udelson JE, Manning WJ. Right ventricular involvement in hypertrophic cardiomyopathy. *Am J Cardiol.* 2007;100:1293-8.

96. Kwon DH, Setser RM, Thamilarasan M, Popovic ZV, Smedira NG, Schoenhagen P, Garcia MJ, Lever HM, Desai MY. Abnormal papillary muscle morphology is independently associated with increased left ventricular outflow tract obstruction in hypertrophic cardiomyopathy. *Heart.* 2008;94:1295-301.

97. Moon JC, McKenna WJ, McCrohon JA, Elliott PM, Smith GC, Pennell DJ. Toward clinical risk assessment in hypertrophic cardiomyopathy with gadolinium cardiovascular magnetic resonance. *J Am Coll Cardiol.* 2003;41:1561-7.

98. Maron MS, Harrigan C, Buros J, Gibson CM, Hanna C, Lesser JR, Udelson JE, Manning WJ, Maron BJ. Clinical profile and significance of delayed enhancement in hypertrophic cardiomyopathy. *Circulation: Heart Failure.* 2008;1:184-91.

99. Moon JC, Reed E, Sheppard MN, Elkington AG, Ho SY, Burke M, Petrou M, Pennell DJ.The histologic basis of late gadolinium enhancement cardiovascular magnetic resonance in hypertrophic cardiomyopathy. *J Am Coll Cardiol.* 2004;43:2260-4.

100. Rubinshtein R, Glockner JF, Ommen SR, Araoz PA, Ackerman MJ, Sorajja P, Bos JM, Tajik AJ, Valeti US, Nishimura RA, Gersh BJ. Characteristics and clinical significance of late gadolinium enhancement by contrast-enhanced magnetic resonance imaging in patients with hypertrophic cardiomyopathy. *Circ Heart Fail.* 2009;3:51-8.

101. Adabag AS, Maron BJ, Appelbaum E, Harrigan CJ, Buros JL, Gibson CM, Lesser JR, Hanna CA, Udelson JE, Manning WJ, Maron MS. Occurrence and frequency of arrhythmias in hypertrophic cardiomyopathy in relation to delayed enhancement on cardiovascular magnetic resonance. *J Am Coll Cardiol.* 2008;51:1369-74.

102. Kwon DH, Setser RM, Popovic ZB, Thamilarasan M, Sola S, Schoenhagen P, Garcia MJ, Flamm SD, Lever HM, Desai MY. Association of myocardial fibrosis, electrocardiography and ventricular tachyarrhythmia in hypertrophic cardiomyopathy: a delayed contrast enhanced MRI study. *Int J Cardiovasc Imaging.* 2008; 24:617-25.

103. Maron BJ, Seidman JG, Seidman CE. Proposal for contemporary screening strategies in families with hypertrophic cardiomyopathy. *J Am Coll Cardiol.* 2004;44:2125-32.

104. Nagueh SF, Kopelen HA, Lim DS, Zoghbi WA, Quinones MA, Roberts R, Marian AJ. Tissue Doppler imaging consistently detects myocardial contraction and

relaxation abnormalities, irrespective of cardiac hypertrophy, in a transgenic rabbit model of human hypertrophic cardiomyopathy. *Circulation.* 2000;102:1346-50.

105. Germans T, Wilde AA, Dijkmans PA, Chai W, Kamp O, Pinto YM, van Rossum AC.Structural abnormalities of the inferoseptal left ventricular wall detected by cardiac magnetic resonance imaging in carriers of hypertrophic cardiomyopathy mutations. *J Am Coll Cardiol*. 2006;48:2518-23.

106. Bos JM, Towbin JA, Ackerman MJ. Diagnostic, prognostic, and therapeutic implications of genetic testing for hypertrophic cardiomyopathy. *J Am Coll Cardiol.* 2009;54:201–11.

107. Wordsworth S, Leal J, Blair E, Legood R, Thomson K, Seller A, Taylor J, Watkins H. DNA testing for hypertrophic cardiomyopathy: a cost-effectiveness model. *Eur Heart J.* 2010;31:926-35.

108. Melacini P, Basso C, Angelini A, Calore C, Bobbo F, Tokajuk B, Bellini N, Smaniotto G, Zucchetto M, Iliceto S, Thiene G and Maron BJ. Clinicopathologic profiles of progressive heart failure in hypertrophic cardiomyopathy. *Eur Heart J.* 2010; 31:2111-23.

109. Sharma S, Elliott P, Whyte G, Jones S, Mahon N, Whipp B, McKenna WJ. Utility of cardiopulmonary exercise in the assessment of clinical determinants of functional capacity in hypertrophic cardiomyopathy. *Am J Cardiol.* 2000;86:162–8.

110. Maron BJ, Olivotto I, Spirito P, Casey SA, Bellone P, Gohman TE, Graham KJ, Burton DA, Cecchi F. Epidemiology of hypertrophic cardiomyopathy-related death: revisited in a large non–referral-based patient population. *Circulation.* 2000;102:858–64.

111. Maron BJ, Roberts WC, Epstein SE. Sudden death in hypertrophic cardiomyopathy: a profile of 78 patients. *Circulation.* 1982;65:1388-94.

112. Maron BJ, Shirani J, Poliac LC, Mathenge R, Roberts WC, Mueller FO. Sudden death in young competitive athletes. Clinical, demographic, and pathological profiles. *JAMA.* 1996;276:199–204.

113. Elliott PM, Poloniecki J, Dickie S, Sharma S, Monserrat L, Varnava A, Mahon NG, McKenna WJ.. Sudden death in hypertrophic cardiomyopathy: identification of high risk patients. *J Am Coll Cardiol.* 2000;36:2212-8.

114. Elliott PM, Gimeno B Jr., Mahon NG, Poloniecki JD, McKenna WJ. Relation between severity of left ventricular hypertrophy and prognosis in patients with hypertrophic cardiomyopathy. *Lancet.* 2001;357:420–4.

115. Priori SG, Aliot E, Blomstrom-Lundqvist C, Bossaert L, Breithardt G, Brugada P, Camm AJ, Cappato R, Cobbe SM, Di Mario C, Maron BJ, McKenna WJ, Pedersen AK, Ravens U, Schwartz PJ, Trusz-Gluza M, Vardas P, Wellens HJ, Zipes DP. Task force on sudden cardiac death of the European Society of Cardiology. *Eur Heart J.* 2001;22:1374–450.

116. Moolman JC, Corfield VA, Posen B, Ngumbela K, Seidman C, Brink PA, Watkins H. Sudden death due to troponin T mutations. *J Am Coll Cardiol.* 1997;29:549-555.

117. Watkins H, McKenna WJ, Thierfelder L, Suk HJ, Anan R, O'Donoghue A, Spirito P, Matsumori A, Moravec CS, Seidman JG, Seidman CE. Mutations in the genes for cardiac troponin T and alpha-tropomyosin in hypertrophic cardiomyopathy. *N Engl J Med.* 1995;332:1058–64.

118. Spirito P, Bellone P, Harris KM, Bernabo P, Bruzzi P, Maron BJ. Magnitude of left ventricular hypertrophy and risk of sudden death in hypertrophic cardiomyopathy. *N Engl J Med.* 2000;342:1778–85.

119. Kofflard MJ, ten Cate FJ, van der Lee C, van Domburg RT. Hypertrophic cardiomyopathy in a large community-based population: clinical outcome and identification of risk factors for sudden cardiac death and clinical deterioration. *J Am Coll Cardiol.* 2003;41:987–93.

120. Yetman AT, McCrindle BW, MacDonald C, Freedom RM, Gow R. Myocardial bridging in children with hypertrophic cardiomyopathy-a risk factor for sudden death. *N Engl J Med.* 1998;339:1201–9.

121. Van Driest SL, Ackerman MJ, Ommen SR, Shakur R, Will ML, Nishimura RA, Tajik AJ, Gersh BJ. Prevalence and severity of “benign” mutations in the beta-myosin heavy chain, cardiac troponin T, and alpha-tropomyosin genes in hypertrophic cardiomyopathy. *Circulation.* 2002;106:3085–90.

122. Christiaans I, Birnie E, Bonsel GJ, Mannens MM, Michels M, Majoor-Krakauer D, Dooijes D, van Tintelen JP, van den Berg MP, Volders PG, Arens YH, van den Wijngaard A, Atsma DE, Helderman-van den Enden AT, Houweling AC, de Boer K,

van der Smagt JJ, Hauer RN, Marcelis CL, Timmermans J, van Langen IM, Wilde AA. Manifest disease, risk factors for sudden cardiac death, and cardiac events in a large nationwide cohort of predictively tested hypertrophic cardiomyopathy mutation carriers: determining the best cardiological screening strategy. *Eur Heart J*. 2011;32:1161-70.

123. Pelliccia A, Fagard R, Bjørnstad HH, Anastassakis A, Arbustini E, Assanelli D, Biffi A, Borjesson M, Carrè F, Corrado D, Delise P, Dorwarth U, Hirth A, Heidbuchel H, Hoffmann E, Mellwig KP, Panhuyzen-Goedkoop N, Pisani A, Solberg EE, van-Buuren F, Vanhees L, Blomstrom-Lundqvist C, Deligiannis A, Dugmore D, Glikson M, Hoff PI, Hoffmann A, Hoffmann E, Horstkotte D, Nordrehaug JE, Oudhof J, McKenna WJ, Penco M, Priori S, Reybrouck T, Senden J, Spataro A, Thiene G. (Study Group of Sports Cardiology of the Working Group of Cardiac Rehabilitation and Exercise Physiology; Working Group of Myocardial and Pericardial Diseases of the European Society of Cardiology). Recommendations for competitive sports participation in athletes with cardiovascular disease: a consensus document from the Study Group of Sports Cardiology of the Working Group of Cardiac Rehabilitation and Exercise Physiology and the Working Group of Myocardial and Pericardial Diseases of the European Society of Cardiology. *Eur Heart J*. 2005;26:1422-45.

124. Maron BJ, Shen W-K, Link MS, Epstein AE, Almquist AK, Daubert JP, Bardy GH, Favale S, Rea RF, Boriani G, Estes NA 3rd, Spirito P. Efficacy of implantable cardioverter-defibrillators for the prevention of sudden death in patients with hypertrophic cardiomyopathy. *N Engl J Med.* 2000;342:365–73.

125. Maron BJ, Spirito P, Shen W-K, Haas TS, Formisano F, Link MS, Epstein AE, Almquist AK, Daubert JP, Lawrenz T, Boriani G, Estes NA 3rd, Favale S, Piccininno M, Winters SL, Santini M, Betocchi S, Arribas F, Sherrid MV, Buja G, Semsarian C, Bruzzi P. Implantable cardioverter-defibrillators and prevention of sudden cardiac death in hypertrophic cardiomyopathy. *JAMA.* 2007;298:405–412.

126. Melacini P, Maron BJ, Bobbo F, Basso C, Tokajuk B, Zucchetto M, Thiene G, Iliceto S. Evidence that pharmacological strategies lack efficacy for the prevention of sudden death in hypertrophic cardiomyopathy. *Heart.* 2007;93:708–710.

127. Pelliccia A, Corrado D, Bjornstad HH, Panhuyzen-Goedkoop N, Urhausen A, Carre F, Anastasakis A, Vanhees L, Arbustini E, Priori S. Recommendations for participation in competitive sport and leisure time physical activity in individuals with

cardiomyopathies, myocarditis and pericarditis. *Eur J Cardiovasc Prev Rehabil.* 2006;13:876-885.

128. McKenna WJ, Behr ER. Hypertrophic cardiomyopathy: management, risk stratification, and prevention of sudden death. *Heart* 2002;87:169–176.

129. Bonow RO, Dilsizian V, Rosing DR, Maron BJ, Bacharach SL, Green MV. Verapamil-induced improvement in left ventricular diastolic filling and increased exercise tolerance in patients with hypertrophic cardiomyopathy: short- and long-term effects. *Circulation.* 1985;72:853–64.

130. Udelson JE, Bonow RO, O'Gara PT, Maron BJ, Van Lingen A, Bacharach SL, Epstein SE. Verapamil prevents silent myocardial perfusion abnormalities during exercise in asymptomatic patients with hypertrophic cardiomyopathy. *Circulation.* 1989;79:1052–60.

131. Fuster V, Rydén LE, Cannom DS, Crijns HJ, Curtis AB, Ellenbogen KA, Halperin JL, Le Heuzey JY, Kay GN, Lowe JE, Olsson SB, Prystowsky EN, Tamargo JL, Wann S; Task Force on Practice Guidelines, American College of Cardiology/American Heart Association; Committee for Practice Guidelines, European Society of Cardiology; European Heart Rhythm Association; Heart Rhythm Society. ACC/AHA/ESC 2006 guidelines for the management of patients with atrial fibrillation-executive summary: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and the European Society of Cardiology Committee for Practice Guidelines (Writing Committee to Revise the 2001 Guidelines for the Management of Patients with Atrial Fibrillation). *Eur Heart J*. 2006;27:1979-2030.

132. McKenna WJ, Harris L, Rowland E, Kleinebenne A, Krikler DM, Oakley CM, Goodwin JF. Amiodarone for longterm management of patients with hypertrophic cardiomyopathy. *Am J Cardiol.* 1984;54:802–10.

133. Spirito P, Rapezzi C, Bellone P, Betocchi S, Autore C, Conte MR, Bezante GP, Bruzzi P. Infective endocarditis in hypertrophic cardiomyopathy: prevalence, incidence, and indications for antibiotic prophylaxis. *Circulation.* 1999;99:2132–7.

134. Wilson W, Taubert KA, Gewitz M, Lockhart PB, Baddour LM, Levison M, Bolger A, Cabell CH, Takahashi M, Baltimore RS, Newburger JW, Strom BL, Tani LY, Gerber M, Bonow RO, Pallasch T, Shulman ST, Rowley AH, Burns JC, Ferrieri P,

Gardner T, Goff D, Durack DT; (American Heart Association Rheumatic Fever, Endocarditis, and Kawasaki Disease Committee; American Heart Association Council on Cardiovascular Disease in the Young; American Heart Association Council on Clinical Cardiology; American Heart Association Council on Cardiovascular Surgery and Anesthesia; Quality of Care and Outcomes Research Interdisciplinary Working Group). Prevention of infective endocarditis: guidelines from the American Heart Association: a guideline from the American Heart Association Rheumatic Fever, Endocarditis, and Kawasaki Disease Committee, Council on Cardiovascular Disease in the Young, and the Council on Clinical Cardiology, Council on Cardiovascular Surgery and Anesthesia, and the Quality of Care and Outcomes Research Interdisciplinary Working Group. *Circulation*. 2007;116:1736-54.

135. Maron BJ, Merrill WH, Freier PA, Kent KM, Epstein SE, Morrow AG. Long-term clinical course and symptomatic status of patients after operation for hypertrophic subaortic stenosis. *Circulation.* 1978;57:1205–13.

136. McCully RB, Nishimura RA, Tajik AJ, Schaff HV, Danielson GK. Extent of clinical improvement after surgical treatment of hypertrophic obstructive cardiomyopathy. *Circulation.* 1996;94:467–71.

137. McIntosh CL, Maron BJ. Current operative treatment of obstructive hypertrophic cardiomyopathy. *Circulation.* 1988;78:487–95.

138. Mohr R, Schaff HV, Danielson GK, Puga FJ, Pluth JR, Tajik AJ. The outcome of surgical treatment of hypertrophic obstructive cardiomyopathy. Experience over 15 years. *J Thorac Cardiovasc Surg.* 1989;97:666–74.

139. Maron BJ, Dearani JA, Ommen SR, Maron MS, Schaff HV, Gersh BJ, Nishimura RA. The case for surgery in obstructive hypertrophic cardiomyopathy. *J Am Coll Cardiol*. 2004;44:2044-53.

140. Ommen SR, Maron BJ, Olivotto I, Maron MS, Cecchi F, Betocchi S, Gersh BJ, Ackerman MJ, McCully RB, Dearani JA, Schaff HV, Danielson GK, Tajik AJ, Nishimura RA. Long-term effects of surgical septal myectomy on survival in patients with obstructive hypertrophic cardiomyopathy. *J Am Coll Cardiol*. 2005;46:470-6.

141. Maron BJ. Controversies in cardiovascular medicine. Surgical myectomy remains the primary treatment option for severely symptomatic patients with obstructive hypertrophic cardiomyopathy. *Circulation*. 2007;116:196-206.

142. Maron BJ, Yacoub M, Dearani JA.Benefits of surgery in obstructive hypertrophic cardiomyopathy: bring septal myectomy back for European patients.*Eur Heart J*. 2011;32:1055-8.

143. Schoendube FA, Klues HG, Reith S, Flachskampf FA, Hanrath P, Messmer BJ. Long-term clinical and echocardiographic follow-up after surgical correction of hypertrophic obstructive cardiomyopathy with extended myectomy and reconstruction of the subvalvular mitral apparatus. *Circulation* 1995;92 Suppl:II122–7.

144. Maron BJ, Nishimura RA, Danielson GK. Pitfalls in clinical recognition and a novel operative approach for hypertrophic cardiomyopathy with severe outflow obstruction due to anomalous papillary muscle. *Circulation.* 1998;98:2505–8.

145. McIntosh CL, Maron BJ, Cannon RO III, Klues HG. Initial results of combined anterior mitral leaflet plication and ventricular septal myotomy-myectomy for relief of left ventricular outflow tract obstruction in patients with hypertrophic cardiomyopathy. *Circulation.* 1992;86 Suppl:II60–7.

146. Theodoro DA, Danielson GK, Feldt RH, Anderson BJ. Hypertrophic obstructive cardiomyopathy in pediatric patients: results of surgical treatment. *J Thorac Cardiovasc Surg.* 1996;112:1589–97.

147. Kuhn H, Gietzen F, Leuner C, Gerenkamp T. Induction of subaortic septal ischaemia to reduce obstruction in hypertrophic obstructive cardiomyopathy. Studies to develop a new catheter-based concept of treatment. *Eur Heart J.* 1997;18:846–51.

148. Maron BJ. Role of alcohol septal ablation in treatment of obstructive hypertrophic cardiomyopathy. *Lancet.* 2000;355:425–6.

149. Mazur W, Nagueh SF, Lakkis NM, Middleton KJ, Killip D, Roberts R, Spencer WH 3rd. Regression of left ventricular hypertrophy after nonsurgical septal reduction therapy for hypertrophic obstructive cardiomyopathy. *Circulation.* 2001;103:1492–6.

150. Gietzen FH, Leuner CJ, Obergassel L, Strunk-Muller C, Kuhn H. Role of transcoronary ablation of septal hypertrophy in patients with hypertrophic cardiomyopathy, NYHA functional class III or IV and outflow obstruction only under provocable conditions. *Circulation.* 2002;106:454–9.

151. Kuhn H, Gietzen F, Leuner C. “The abrupt no-flow:” a no-reflow like phenomenon in hypertrophic cardiomyopathy. *Eur Heart J.* 2002;23:91–3.

152. Firoozi S, Elliott PM, Sharma S, et al. Septal myotomy-myectomy and transcoronary septal alcohol ablation in hypertrophic obstructive cardiomyopathy: A comparison of clinical, hemodynamic and exercise outcomes. *Eur Heart J.* 2002;20:1617–24.

153. Olivotto I, Ommen SR, Maron MS, Cecchi F, Maron BJ Surgical myectomy versus alcohol septal ablation for obstructive hypertrophic cardiomyopathy. Will there ever be a randomized trial? *J Am Coll Cardiol*. 2007;50:831-4

154. Fananapazir L, Epstein ND, Curiel RV, Panza JA, Tripodi D, McAreavey D. Long-term results of dual-chamber (DDD) pacing in obstructive hypertrophic cardiomyopathy. Evidence for progressive symptomatic and hemodynamic improvement and reduction of left ventricular hypertrophy. *Circulation.* 1994;90:2731–42.

155. Posma JL, Blanksma PK, Van Der Wall EE, Vaalburg W, Crijns HJ, Lie KI. Effects of permanent dual chamber pacing on myocardial perfusion in symptomatic hypertrophic cardiomyopathy. *Heart.* 1996; 76:358–62.

156. Maron BJ, Nishimura RA, McKenna WJ, Rakowski H, Josephson ME, Kieval RS. Assessment of permanent dual-chamber pacing as a treatment for drug-refractory symptomatic patients with obstructive hypertrophic cardiomyopathy. A randomized, double-blind, crossover study (M-PATHY). *Circulation.* 1999;99:2927–33.

157. Nishimura RA, Trusty JM, Hayes DL, Ilstrup DM, Larson DR, Hayes SN, Allison TG, Tajik AJ.. Dual-chamber pacing for hypertrophic cardiomyopathy: a randomized, double-blind, crossover trial. *J Am Coll Cardiol.* 1997;29:435–41.

158. Jarcho JA, McKenna W, Pare JA, Solomon SD, Holcombe RF, Dickie S, Levi T, Donis-Keller H, Seidman JG, Seidman CE. Mapping a gene for familial hypertrophic cardiomyopathy to chromosome 14q1. *N Engl J Med.* 1989;321:1372–1378.

159. Saez LJ, Gianola KM, McNally EM, Feghali R, Eddy R, Shows TB, Leinwand LA. Human cardiac myosin heavy chain genes and their linkage in the genome. *Nucleic Acids Res*. 1987;15:5443–5459.

160. Watkins H, Rosenzweig A, Hwang DS, Levi T, McKenna W, Seidman CE, Seidman JG. Characteristics and prognostic implications of myosin missense mutations in familial hypertrophic cardiomyopathy. *N Engl J Med*. 1992;326:1108 –1114.

161. Marian AJ, Yu QT, Mares A Jr, Hill R, Roberts R, Perryman MB. Detection of a new mutation in the beta-myosin heavy chain gene in an individual with hypertrophic cardiomyopathy. *J Clin Invest*. 1992;90:2156–2165.

162. Dufour C, Dausse E, Fetler L, Dubourg O, Bouhour JB, Vosberg HP, Guicheney P, Komajda M, Schwartz K. Identification of a mutation near a functional site of the beta cardiac myosin heavy chain gene in a family with hypertrophic cardiomyopathy. *J Mol Cell Cardiol*. 1994;26:1241–1247.

163. Watkins H, MacRae C, Thierfelder L, Chou YH, Frenneaux M, McKenna W, Seidman JG, Seidman CE. A disease locus for familial hypertrophic cardiomyopathy maps to chromosome 1q3. *Nat Genet*. 1993;3:333–337.

164. Thierfelder L, Watkins H, MacRae C, Lamas R, McKenna W, Vosberg HP, Seidman JG, Seidman CE. Alpha-tropomyosin and cardiac troponin T mutations cause familial hypertrophic cardiomyopathy: a disease of the sarcomere. *Cell*. 1994;77:701–712.

165. Schleef M, Werner K, Satzger U, Kaupmann K, Jockusch H. Chromosomal localization and genomic cloning of the mouse alphatropomyosin gene Tpm-1. *Genomics*. 1993;17:519 –521.

166. Seidman CE and Seidman JG. Identifying sarcomere gene mutations in hypertrophic cardiomyopathy: a personal history. *Circ Res.* 2011;108:743-750.

167. Kamisago M, Sharma SD, DePalma SR, Solomon S, Sharma P, McDonough B, Smoot L, Mullen MP, Woolf PK, Wigle ED, Seidman JG, Seidman CE. Mutations in sarcomere protein genes as a cause of dilated cardiomyopathy. *N Engl J Med.* 2000;343:1688 –96.

168. Mohapatra B, Jimenez S, Lin JH, Bowles KR, Coveler KJ, Marx JG, Chrisco MA, Murphy RT, Lurie PR, Schwartz RJ, Elliott PM, Vatta M, McKenna W, Towbin JA, Bowles NE. Mutations in the muscle LIM protein and alpha-actinin-2 genes in dilated cardiomyopathy and endocardial fibroelastosis. *Mol Genet Metab.* 2003;80:207–15.

169. Hayashi T, Arimura T, Itoh-Satoh M, Ueda K, Hohda S, Inagaki N, Takahashi M, Hori H, Yasunami M, Nishi H, Koga Y, Nakamura H, Matsuzaki M, Choi BY, Bae SW, You CW, Han KH, Park JE, Knöll R, Hoshijima M, Chien KR, Kimura A. Tcap gene mutations in hypertrophic cardiomyopathy and dilated cardiomyopathy. *J Am Coll Cardiol.* 2004;44:2192–201.

170. Vasile VC, Will ML, Ommen SR, Edwards WD, Olson TM, Ackerman MJ. Identification of a metavinculin missense mutation, R975W, associated with both hypertrophic and dilated cardiomyopathy. *Mol Genet Metab.* 2006;87:169 –74.

171. Vasile VC, Ommen SR, Edwards WD, Ackerman MJ. A missense mutation in a ubiquitously expressed protein, vinculin, confers susceptibility to hypertrophic cardiomyopathy. *Biochem Biophys Res Commun.* 2006;345:998 –1003.

172. Osio A, Tan L, Chen SN, Lombardi R, Nagueh SF, Shete S, Roberts R, Willerson JT, Marian AJ. Myozenin 2 is a novel gene for human hypertrophic cardiomyopathy. *Circ Res.* 2007;100:766–8.

173. Minamisawa S, Sato Y, Tatsuguchi Y, Fujino T, Imamura S, Uetsuka Y, Nakazawa M, Matsuoka R. Mutation of the phospholamban promoter associated with hypertrophic cardiomyopathy. *Biochem Biophys Res Commun.* 2003;304:1– 4.

174. Haghighi K, Kolokathis F, Gramolini AO, Waggoner JR, Pater L, Lynch RA, Fan GC, Tsiapras D, Parekh RR, Dorn GW 2nd, MacLennan DH, Kremastinos DT, Kranias EG. A mutation in the human phospholamban gene, deleting arginine 14, results in lethal, hereditary cardiomyopathy. *Proc Natl Acad Sci U S A.* 2006;103:1388–93.

175. Landstrom AP, Weisleder N, Batalden KB, Bos JM, Tester DJ, Ommen SR, Wehrens XH, Claycomb WC, Ko JK, Hwang M, Pan Z, Ma J, Ackerman MJ. Mutations in JPH2- encoded junctophilin-2 associated with hypertrophic cardiomyopathy in humans. *J Moll Cell Cardiol.* 2007;42:1026 –35.

176. Chiu C, Tebo M, Ingles J, Yeates L, Arthur JW, Lind JM, Semsarian C. Genetic screening of calcium regulation genes in familial hypertrophic cardiomyopathy. *J Mol and Cell Cardiol.* 2007;43:337-343.

177. Chiu C, Bagnall RD, Ingles J, Yeates L, Kennerson M, Donald JA, Jormakka M, Lind JM, Semsarian C. Mutations in alpha-actinin-2 cause hypertrophic cardiomyopathy: a genome-wide analysis. *J Am Coll Cardiol*. 2010;55:1127–1135.

178. Marian AJ, Roberts R. The molecular genetic basis for hypertrophic cardiomyopathy. *J Mol Cell Cardiol*. 2001;33:655–70.

179. Niimura H, Patton KK, McKenna WJ, Soults J, Maron BJ, Seidman JG, Seidman CE. Sarcomere protein gene mutations in hypertrophic cardiomyopathy of the elderly. *Circulation.* 2002;105:446–51.

180. Richard P, Charron P, Carrier L, Ledeuil C, Cheav T, Pichereau C, Benaiche A, Isnard R, Dubourg O, Burban M, Gueffet JP, Millaire A, Desnos M, Schwartz K, Hainque B, Komajda M; EUROGENE Heart Failure Project. Hypertrophic cardiomyopathy. Distribution of disease genes, spectrum of mutations, and implications for a molecular diagnosis strategy. *Circulation.* 2003; 107:2227-32.

181. Van Driest SL, Ommen SR, Tajik AJ, Gersh BJ, Ackerman MJ. Yield of genetic testing in hypertrophic cardiomyopathy. *Mayo Clin Proc.* 2005;80:739–44.

182. Gollob MH, Green MS, Tang AS, Gollob T, Karibe A, Ali Hassan AS, Ahmad F, Lozado R, Shah G, Fananapazir L, Bachinski LL, Roberts R. Identification of a gene responsible for familial Wolff-Parkinson-White syndrome. *N Engl J Med.* 2001;344:1823–31.

183. Blair E, Redwood C, Ashrafian H, Oliveira M, Broxholme J, Kerr B, Salmon A, Ostman-Smith I, Watkins H. Mutations in the gamma(2) subunit of AMP-activated protein kinase cause familial hypertrophic cardiomyopathy: evidence for the central role of energy compromise in disease pathogenesis. *Hum Mol Genet.* 2001;10:1215–20.

184. Arad M, Benson DW, Perez-Atayde AR, McKenna WJ, Sparks EA, Kanter RJ, McGarry K, Seidman JG, Seidman CE. Constitutively active AMP kinase mutations cause glycogen storage disease mimicking hypertrophic cardiomyopathy. *J Clin Invest.* 2002;109:357– 62.

185. Arad M, Maron BJ, Gorham JM, Johnson WH Jr, Saul JP, Perez-Atayde AR, Spirito P, Wright GB, Kanter RJ, Seidman CE, Seidman JG. Glycogen storage diseases presenting as hypertrophic cardiomyopathy. *N Engl J Med.* 2005;352:362–72.

186. Yang Z, McMahon CJ, Smith LR, Bersola J, Adesina AM, Breinholt JP, Kearney DL, Dreyer WJ, Denfield SW, Price JF, Grenier M, Kertesz NJ, Clunie SK, Fernbach SD, Southern JF, Berger S, Towbin JA, Bowles KR, Bowles NE. Danon disease as an underrecognized cause of hypertrophic cardiomyopathy in children. *Circulation.* 2005;112:1612–7.

187. Fanin M, Nascimbeni AC, Fulizio L, Spinazzi M, Melacini P, Angelini C. Generalized lysosome-associated membrane protein-2 defect explains multisystem clinical involvement and allows leukocyte diagnostic screening in Danon disease. *Am J Pathol.* 2006;168:1309-20.

188. Watkins H, Seidman CE, Seidman JG, Feng HS, Sweeney HL. Expression and functional assessment of a truncated cardiac troponin T that causes hypertrophic cardiomyopathy. Evidence for a dominant negative action. *J Clin Invest.* 1996;98:2456-2461.

189. Roopnarine O, Leinwand LA. Functional analysis of myosin mutations that cause familial hypertrophic cardiomyopathy. *Biophys J.* 1998;75:3023-3030.

190. Sata M, Ikebe M. Functional analysis of the mutations in the human cardiac beta-myosin that are responsible for familial hypertrophic cardiomyopathy. Implication for the clinical outcome. *J Clin Invest.* 1996;98:2866-2873.

191. Tardiff JC. Sarcomeric proteins and familial hypertrophic cardiomyopathy. Linking mutations in structural proteins to complex cardiovascular phenotypes. *Heart Fail Rev.* 2005;10:237-248.

192. Geisterfer-Lowrance AA, Christe M, Conner DA, Ingwall JS, Schoen FJ, Seidman CE, Seidman JG: A mouse model of familial hypertrophic cardiomyopathy. *Science.* 1996;272:731-734.

193. Marian AJ, Wu Y, Lim DS, McCluggage M, Youker K, Yu QT, Brugada R, DeMayo F, Quinones M, Roberts R. A transgenic rabbit model for human hypertrophic cardiomyopathy. *J Clin Inves.t* 1999;104:1683-1692.

194. Yang Q, Sanbe A, Osinska H, Hewett TE, Klevitsky R, Robbins J. A mouse model of myosin binding protein C human familial hypertrophic cardiomyopathy. *J Clin Invest.* 1998;102:1292-1300.

195. Tardiff JC, Hewett TE, Palmer BM, Olsson C, Factor SM, Moore RL, Robbins J, Leinwand LA. Cardiac troponin T mutations result in allele-specific phenotypes in a mouse model for hypertrophic cardiomyopathy. *J Clin Invest.* 1999;104:469-481.

196. Tyska MJ, Hayes E, Giewat M, Seidman CE, Seidman JG, Warshaw DM. Single-molecule mechanics of R403Q cardiac myosin isolated from the mouse model of familial hypertrophic cardiomyopathy. *Circ Res.* 2000;86:737-744.

197. Palmiter KA, Tyska MJ, Haeberle JR, Alpert NR, Fananapazir L, Warshaw DM. R403Q and L908V mutant beta-cardiac myosin from patients with familial hypertrophic cardiomyopathy exhibit enhanced mechanical performance at the single molecule level. *J Muscle Res Cell Motil.* 2000;21:609-620.

198. Keller DI, Coirault C, Rau T, Cheav T, Weyand M, Amann K, Lecarpentier Y, Richard P, Eschenhagen T, Carrier L. Human homozygous R403W mutant cardiac myosin presents disproportionate enhancement of mechanical and enzymatic properties. *J Mol Cell Cardiol.* 2004;36:355-362.

199. Alcalai R, Seidman JG, Seidman C. Genetic Basis of Hypertrophic Cardiomyopathy:From Bench to the Clinics. *J Cardiovasc Electrophysiol*. 2008;19:104-110.

200. Watkins H. Genetic clues to disease pathways in hypertrophic and dilated cardiomyopathies. *Circulation.* 2003; 107: 1344–46.

201. DiMauro S, Schon EA. Mitochondrial Respiratory-Chain Diseases. *N Engl J Med.* 2003; 348: 2656–68.

202. Holmgren D, Wahlander H, Eriksson BO, Oldfors A, Holme E, Tulinius M. Cardiomyopathy in children with mitochondrial disease; clinical course and cardiological findings. *Eur Heart J.* 2003; 24: 280–88.

203. Watkins H, Ashrafian H, Redwood C. Inherited cardiomyopathies. *N Engl J Med*. 2011 28;364:1643-56.

204. Ashrafian H, Watkins H. Reviews of translational medicine and genomics in cardiovascular disease: new disease taxonomy and therapeutic implications cardiomyopathies: therapeutics based on molecular phenotype. *J Am Coll Cardiol*. 2007;49:1251-64.

205. Crilley JG, Boehm EA, Blair E, Rajagopalan B, Blamire AM, Styles P, McKenna WJ, Ostman-Smith I, Clarke K, Watkins H. Hypertrophic cardiomyopathy due to sarcomeric gene mutations is characterized by impaired energy metabolism irrespective of the degree of hypertrophy. *J Am Coll Cardiol.* 2003; 41: 1776–82.

206. Arad M, Seidman JG, Seidman CE: Phenotypic diversity in hypertrophic cardiomyopathy. *Hum Mol Genet.* 2002;11:2499-2506.

207. Marian AJ, Roberts R. Molecular genetic basis of hypertrophic cardiomyopathy: Genetic markers for sudden cardiac death. *J Cardiovasc Electrophysiol.* 1998;9:88-99.

208. Arad M, Penas-Lado M, Monserrat L, Maron BJ, Sherrid M, Ho CY, Barr S, Karim A, Olson TM, Kamisago M, Seidman JG, Seidman CE. Gene mutations in apical hypertrophic cardiomyopathy. *Circulation.* 2005;112:2805-2811.

209. Mohiddin SA, Begley DA, McLam E, Cardoso JP, Winkler JB, Sellers JR, Fananapazir L. Utility of genetic screening in hypertrophic cardiomyopathy: Prevalence and significance of novel and double (homozygous and heterozygous) beta-myosin mutations. *Genet Test.* 2003;7:21-27.

210. Marian AJ, Yu QT, Workman R, Greve G, Roberts R. Angiotensin converting enzyme polymorphism in hypertrophic cardiomyopathy and sudden cardiac death. *Lancet.* 1993;342:1085-1086.

211. Brugada R, KelseyW, Lechin M, Zhao G, Yu QT, Zoghbi W, Quinones M, Elstein E, Omran A, Rakowski H, Wigle D, Liew CC, Sole M, Roberts R, Marian AJ. Role of candidate modifier genes on the phenotypic expression of hypertrophy in patients with hypertrophic cardiomyopathy. *J Investig Med.* 1997;45:542-551.

212. Lechin M, Quinones MA, Omran A, Hill R, Yu QT, Rakowski H, Wigle D, Liew CC, Sole M, Roberts R, Marian AJ: Angiotensin-I converting enzyme genotypes and left ventricular hypertrophy in patients with hypertrophic cardiomyopathy. *Circulation.* 1995;92:1808-1812.

213. Ortlepp JR, Vosberg HP, Reith S, Ohme F, Mahon NG, Schröder D, Klues HG, Hanrath P, McKenna WJ. Genetic polymorphisms in the renin-angiotensin-aldosterone system associated with expression of left ventricular hypertrophy in hypertrophic cardiomyopathy: a study of five polymorphic genes in a family with a disease causing mutation in the myosin binding protein C gene. *Heart.* 2002;87:270–5.

214. Perkins MJ, Van Driest SL, Ellsworth EG, Will ML, Gersh BJ, Ommen SR, Ackerman MJ. Gene-specific modifying effects of pro-LVH polymorphisms involving the reninangiotensin-aldosterone system among 389 unrelated patients with hypertrophic cardiomyopathy. *Eur Heart J.* 2005;26:2457– 62.

215. Lind JM, Chiu C, Ingles J, Yeates L, Humphries SE, Heather AK, Semsarian C. Sex hormone receptor gene variation associated with phenotype in male hypertrophic cardiomyopathy patients. *J Mol Cell Cardiol.* 2008;45:217–22.

216. Bos JM, Theis JL, Tajik AJ, Gersh BJ, Ommen SR, Ackerman MJ. Relationship between sex, shape, and substrate in hypertrophic cardiomyopathy. *Am Heart J.* 2008;155:1128 –34.

217. Olivotto I, Maron MS, Adabag AS, Casey SA, Vargiu D, Link MS, Udelson JE, Cecchi F, Maron BJ. Gender-related differences in the clinical presentation and outcome of hypertrophic cardiomyopathy. *J Am Coll Cardiol.* 2005;46:480 –7.

218. Daw EW, Chen SN, Czernuszewicz G, Lombardi R, Lu Y, Ma J, Roberts R, Shete S, Marian AJ. Genome-wide mapping of modifier chromosomal loci for human hypertrophic cardiomyopathy. *Hum Mol Genet.* 2007;16:2463–71.

219. Konhilas JP, Watson PA, Maass A, Boucek DM, Horn T, Stauffer BL, Luckey SW, Rosenberg P, Leinwand LA. Exercise can prevent and reverse the severity of hypertrophic cardiomyopathy. *Circ Res.* 2006;98:540-548.

220. Stauffer BL, Konhilas JP, Luczak ED, Leinwand LA. Soy diet worsens heart disease in mice. *J Clin Invest.* 2006;116:209-216.

221. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cell. *Nucleic Acid Res.* 1988; 16:1215.

222. Oefner PJ and Underhill PA. Comparative DNA sequencing by denaturing high-performance liquid chromatography (DHPLC). *Am J Hum Genet.* 1995;57:A266.

223. Fokstuen S, Lyle R, Munoz A, et al. A DNA Resequencing Array for Pathogenic Mutation Detection in Hypertrophic Cardiomyopathy. *Hum Mutat.* 2008; 29:879-85.

224. Fokstuen S, Munoz A, Melacini P, Iliceto S, Perrot A, Ozcelik C, Jeanrenaud X, Rieubland C, Farr M, Faber L, Sigwart U, Mach F, Lerch R, Antonarakis SE, Blouin JL. Rapid detection of genetic variants in hypertrophic cardiomyopathy by custom DNA resequencing array in clinical practice. *J Med Genet.* 2011 Jan 14. [Epub ahead of print]

225. Cutler DJ, Zwick ME, Carrasquillo MM, Yohn CT, Tobin KP, Kashuk C, Mathews DJ, Shah NA, Eichler EE, Warrington JA, Chakravarti A. High-throughput variation detection and genotyping using microarrays. *Genome Res.* 2001;11:1913–1925.

226. Hacia JG. Resequencing and mutational analysis using oligonucleotide microarrays. *Nat Genet.* 1999;21:42–47.

227. Warrington JA, Shah NA, Chen X, Warrington JA, Shah NA, Chen X. New developments in high-throughput resequencing and variation detection using high density microarrays. *Hum Mutat.* 2002;19:402–409.

228. Perrot A, Dietz R, Osterziel KJ. Is there a common genetic basis for all familial cardiomyopathy? *Eur J Heart Failure.* 2007;9:4-6.

229. Geier C, Perrot A, Ozcelik C, Binner P, Counsell D, Hoffmann K, Pilz B, Martiniak Y, Gehmlich K, van der Ven PF, Fürst DO, Vornwald A, von Hodenberg E, Nürnberg P, Scheffold T, Dietz R, Osterziel KJ Mutation in the human muscle LIM protein gene in families with hypertrophic cardiomyopathy. *Circulation.* 2003; 107:1390-1395.

230. Kimura A, Ito-Satoh M, Hayashi T, Takahashi M, Arimura T. Molecular etiology of idiopathic cardiomyopathy in Asian populations. *J Cardiol*. 2001;37:139-46.

231. Anan R, Greve G, Thierfelder L, Watkins H, McKenna WJ, Solomon S, Vecchio C, Shono H, Nakao S, Tanaka H. Prognostic implications of novel beta cardiac myosin heavy chain gene mutations that cause familial hypertrophic cardiomyopathy. *J Clin Invest*. 1994;93:280-5.

232. Consevage MW, Salada GC, Baylen BG, Ladda RL, Rogan PK. A new missense mutation, Arg719Gln, in the beta-cardiac heavy chain myosin gene of patients with familial hypertrophic cardiomyopathy. *Hum Mol Genet*. 1994;3:1025-6.

233. Girolami F, Ho CY, Semsarian C, Baldi M, Will ML, Baldini K, Torricelli F, Yeates L, Cecchi F, Ackerman MJ, Olivotto I. Clinical features and outcome of hypertrophic cardiomyopathy associated with triple sarcomere protein gene mutations. *J Am Coll Cardiol.* 2010;55:1444-53.

234. Nanni L, Pieroni M, Chimenti C, Simionati B, Zimbello R, Maseri A, Frustaci A, Lanfranchi G. Hypertrophic cardiomyopathy: two homozygous cases with "typical" hypertrophic cardiomyopathy and three new mutations in cases with progression to dilated cardiomyopathy. *Biochem Biophys Res Commun*. 2003;309:391-8.

235. Koga Y, Toshima H, Kimura A, Harada H, Koyanagi T, Nishi H, Nakata M, Imaizumi T. Clinical manifestations of hypertrophic cardiomyopathy with mutations in the cardiac beta-myosin heavy chain gene or cardiac troponin T gene. *J Card Fail*. 1996;2:S97-103.

236. Varnava A, Baboonian C, Davison F, de Cruz L, Elliott PM, Davies MJ, McKenna WJ. A new mutation of the cardiac troponin T gene causing familial hypertrophic cardiomyopathy without left ventricular hypertrophy. *Heart*. 1999;82:621-4.

237. Olivotto I, Girolami F, Ackerman MJ, Nistri S, Bos JM, Zachara E, Ommen SR, Theis JL, Vaubel RA, Re F, Armentano C, Poggesi C, Torricelli F, Cecchi F. Myofilament protein gene mutation screening and outcome of patients with hypertrophic cardiomyopathy. *Mayo Clin Proc.* 2008;83:630–8.

238. Carrier L, Bonne G, Bährend E, Yu B, Richard P, Niel F, Hainque B, Cruaud C, Gary F, Labeit S, Bouhour JB, Dubourg O, Desnos M, Hagège AA, Trent RJ, Komajda M, Fiszman M, Schwartz K. Organization and sequence of human cardiac myosin binding protein C gene (MYBPC3) and identification of mutations predicted to produce truncated proteins in familial hypertrophic cardiomyopathy. *Circ Res*. 1997;8:427-34.

239. Van Driest SL, Jaeger MA, Ommen SR, et al. Comprehensive analysis of the beta-myosin heavy chain gene in 389 unrelated patients with hypertrophic cardiomyopathy. *J Am Coll Cardiol*. 2004;44:602–10.

240. Maron BJ and Semsarian C. Emergence of gene mutation carriers and the expanding disease spectrum of hypertrophic cardiomyopathy. *Eur Heart J*. 2010 31:1551-3.

241. Christiaans I, Lakanne dit Deprez RH, van Langene IM, Wilde AA. Ventricular fibrillation in MYH7-related hypertrophic cardiomyopathy before onset of ventricular hypertrophy. *Heart Rhythm.* 2009;6:1366–1369.

242. Ho CY. Genetics and Clinical Destiny: Improving Care in Hypertrophic Cardiomyopathy. *Circulation.* 2010;122;2430-2440.

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