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Hypertrophic cardiomyopathy: the genetic determinants of clinical disease expression

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SUMMARY

Hypertrophic cardiomyopathy (HCM), defined clinically by the presence of unexplained left ventricular hypertrophy, is the most common inherited cardiac disorder. This condition is the major cause of sudden death in the young (<30 years of age) and in athletes. The clinical phenotype is heterogeneous, and mutations in a number of sarcomeric contractile-protein genes are responsible for causing the disease in approximately 60% of individuals with HCM. Other inherited syndromes, as well as metabolic and mitochondrial disorders, can present as clinical phenocopies and can be distinguished by their associated cardiac and noncardiac features and on the basis of their unique molecular genetics. The mode of inheritance, natural history and treatment of phenocopies can differ from those of HCM caused by mutations in sarcomere genes. Detailed clinical evaluation and mutation analysis are, therefore, important in providing an accurate diagnosis in order to enable genetic counseling, prognostic evaluation and appropriate clinical management. This Review summarizes current knowledge on the genetics, disease mechanisms, and correlations between phenotype and genotype in patients with HCM, and discusses the implications of genetic testing in routine clinical practice.

KEYWORDS hypertrophic cardiomyopathy, genetic testing, mutations, phenocopies, sarcomere

REVIEW CRITERIA

This article was based on a comprehensive review of articles on hypertrophic cardiomyopathy published up to June 2007 available on PubMed, and of our clinical and laboratory experience with hypertrophic cardiomyopathy and its genetic causes. The search terms used were: "hypertrophic cardiomyopathy", "sarcomere" and "mutation". Selected papers were full-text publications in the English language. We also expanded our search using cited references within such publications.

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INTRODUCTION

Hypertrophic cardiomyopathy (HCM), defined by the presence of unexplained left ventricular hypertrophy (LVH),[1] affects 1 in 500 people[2]—a prevalence similar to familial hypercholesterolemia.[3,4] In most cases, diagnosis relies on electrocardiographic and echocardiographic demonstration of LVH. Diagnostic criteria are specific in probands but less stringent within the context of familial disease, in which the probability of being affected is closer to 1:2 than 1:500.[5] Hypertrophy secondary to infiltrative (e.g. amyloidosis) and systemic (e.g. systemic hypertension) diseases is usually excluded from HCM diagnostic criteria. HCM is a major cause of premature sudden cardiac death (SCD), particularly in young individuals and in apparently healthy athletes.[6] The current annual frequencies of HCM-related sudden death are approximately 1–2% in children and adolescents, and 0.5–1% in adults.[7,8] The disease has variable penetrance, age of onset and distribution, and is associated with differing hemodynamics, degrees of hypertrophy, clinical presentation and prognosis. Clinical presentation typically includes chest pain, exertion-related dyspnea, or impaired consciousness. The majority of individuals with HCM remain asymptomatic, but others experience progressive exercise intolerance and heart failure symptoms, or a sentinel unexpected SCD, which can occur at any age.[6–10]

LVH in HCM is usually diffuse and maximal in the interventricular septum, but localized forms involving other segments of the left and right ventricle have been documented.[9,10] The characteristic histopathological hallmarks of HCM include myocyte disarray, and myofibrillar disorganization with interstitial and replacement fibrosis.[9,11,12] Myocyte disarray can be present in up to 40% of the right and left ventricle and is associated with premature sudden death.[13]

In this Review we examine the genetics of HCM, the possible disease mechanisms, and the phenotype–genotype correlations. We also discuss the implications of genetic testing in routine clinical practice.

GENETIC BASIS OF HYPERTROPHIC CARDIOMYOPATHY

HCM is a genetically heterogeneous condition, usually inherited as an autosomal dominant trait.[9,14] Molecular genetic studies have defined HCM as a disease of the sarcomere, the contractile unit within the cardiac myocyte that is comprised of thick and thin filaments (Figure 1).[11,12,15] Mutations in genes encoding sarcomeric proteins can impair normal protein function, leading to the morphological and physiological manifestations observed in HCM.

The precise mechanisms by which sarcomere gene mutations lead to HCM remain incompletely understood. There are data to support a 'poison peptide' (or dominant–negative) action of the affected allele on the function of the wild-type allele as the predominant mechanism. Another possible mechanism is 'haploinsufficiency', in which there is insufficient protein produced by the normal allele, leading to an imbalance in sarcomere protein stoichiometry.[11–13,16] Experimental studies have revealed functional defects of the sarcomere such as mutation-dependent decreases or increases in force generation, disturbances in Ca^{2+} cycling and abnormal ATP use.[11,12,15,17,18] Of note, in both animal models of HCM and humans, diastolic dysfunction precedes the development of hypertrophy.[19,20] Ongoing research in animal models is targeting abnormal calcium handling and examining the effect of treatments on the development of myocyte disarray and fibrosis.[21,22]

The first gene mutation to be identified and linked to a cardiac disease was a missense mutation in the β-myosin heavy chain gene, which cosegregated with disease in a large French Canadian family with HCM.[23,24] To date, over 450 mutations in 20 sarcomere-related and myofilament-related genes have been identified in HCM (Table 1).[25–38] Mutations in the genes for β-myosin heavy chain (*MYH7*), myosin-binding protein C (*MYBPC3*), and cardiac troponin I (*TNNI3*) and troponin T (*TNNT2*) account for the majority of reported genotyped cases.[9,11–13,15,17,18,24–34] Estimates of the prevalence of mutations in these genes vary, in part due to differences in the populations studied; mutations in *MYH7* are found in approximately 30% of individuals with HCM, *MYBPC3* in approximately 20%, and *TNNT2* and *TNNI3* in 3–5%.[9,17,18,39] Reported pathogenic sequence changes are mainly missense, nonsense and frameshift mutations, with a few in-frame deletions and insertions. In individuals with *MYH7*-related HCM more than 90% of mutations are missense, but in those with *MYBPC3*-related HCM frameshift and nonsense mutations predominate.[17]

Mutations in sarcomeric genes account for approximately 60% of all cases of HCM.[15,39] This relatively low percentage underscores a potential limitation of clinical mutation analysis. The absence of sarcomeric gene mutations in the remaining HCM population could be partially a result of shortcomings in current mutation-detection methods and strategies, or because patients have disease-causing mutations in as yet unidentified sarcomere genes. Recent reports have suggested that mutations in genes encoding sarcomere-associated proteins—such as myosin light chain kinase,[40] muscle LIM protein,[41] LIM binding domain 3,[42] telethonin,[43] vinculin and metavinculin,[44,45] α-actinin 2,[42] phospholamban,[46] myozenin 2,[47] and junctophilin 2[48]—could also be associated with the HCM phenotype. Mutations in these genes seem to be rare.

Another possible explanation for the low proportion of cases thought to be caused by sarcomere gene mutations is that the HCM population without sarcomeric gene mutations could have one of several diseases that mimic the phenotypic expression of HCM (i.e. phenocopies of the disease [Table 2]). These phenocopies include various types of metabolic disease (e.g. glycogen storage disease II and III),[49] Anderson–Fabry disease,[50] mitochondrial diseases,[51] syndromes (e.g. Noonan and LEOPARD syndromes),[52,53] Friedreich's ataxia[54] and other rare diseases.[55,56] A mutation in a nonsarcomeric gene, the γ^2 regulatory subunit of the AMP-activated protein kinase (*PRKAG2*), causes a glycogen storage disorder that mimics HCM, Wolff–Parkinson–White syndrome and progressive conduction disease.[55] Another metabolic phenocopy of HCM that has been associated with Wolff–Parkinson–White syndrome is Danon disease, which is caused by a mutation in lysosome-associated membrane protein 2 (*LAMP2*).[56] HCM secondary to sarcomere gene mutations has to be distinguished from phenocopies because important differences exist between the two conditions in clinical course, management and prognosis.

Genotype–phenotype correlations in sarcomeric hypertrophic cardiomyopathy

Initial genetic studies in HCM attempted to find associations between particular gene defects and

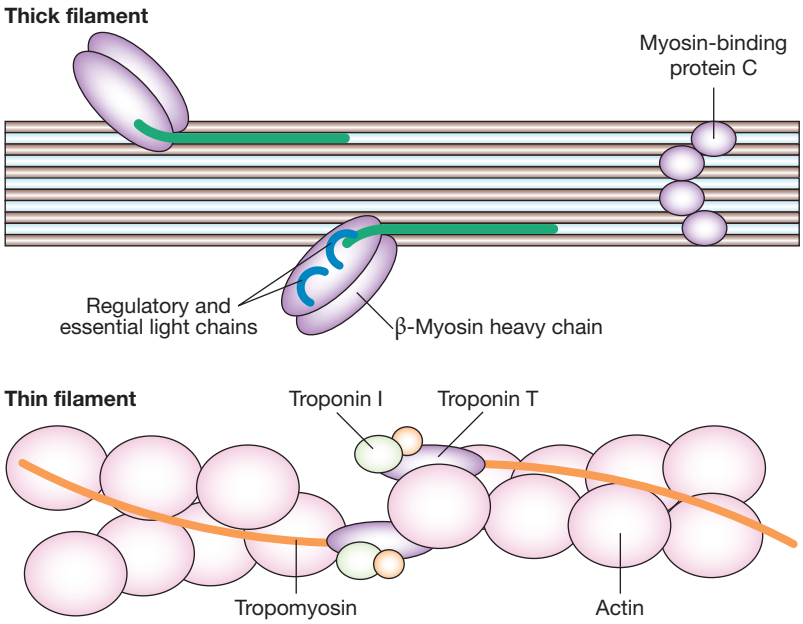

Figure 1 Schematic diagram of the cardiac sarcomere. The sarcomere, a fundamental structural and functional unit of the heart muscle, is composed of the following contractile proteins: myosin-binding protein C, regulatory and essential light chains, and β-myosin heavy chain (thick filament), and actin, tropomyosin, troponin I, and troponin T (thin filament). Current concepts suggest that muscle contraction is achieved by the sliding and interdigitating of the thick and thin filaments. This mechanism is dependent on complex interactions between sarcomeric proteins and is regulated by calcium via the troponin–tropomyosin complex. Hypertrophic cardiomyopathy can be caused by mutations in genes coding for these proteins.

specific clinical phenotypes. Several genotype–phenotype concepts were proposed, mainly for HCM caused by *MYH7*, *MYBPC3* and *TNNT2*.

β-Myosin heavy chain

MYH7 mutations are usually associated with moderate to severe hypertrophy, high disease penetrance[25] and variable prognosis. The Arg403Gln and Arg453Cys mutations cause severe disease with high penetrance (~100%), severe hypertrophy, and high rates of sudden death and other disease-related complications.[25] By contrast, the Val606Met mutation in the same gene is associated with moderate LVH but good prognosis. Investigators proposed that improvement in prognosis was related to the difference in electric charge of the substituted amino acid relative to the original amino acid,[25] but this observation has not yet been confirmed.[28] More recently, it has been suggested that the prognosis of patients with *MYH7* mutations is directly related to which functional domain of the β-myosin protein is affected.[26] With regards to *MYH7*, however, variability is normal; mutations in the same domain can cause markedly different phenotypes (e.g. Arg403Gln and Phe513Cys mutations in the head domain of *MYH7* lead to a very severe and a more benign phenotype, respectively).[25,57]

Not all families with *MYH7* mutations have high penetrance and moderate to severe hypertrophy. Some reports have analyzed large families with *MYH7*-related HCM who have low penetrance, mild hypertrophy and good prognosis, and in whom the majority of gene carriers do not fulfill echocardiographic diagnostic criteria.[58,59] The majority of individuals in these families would not have been recognized as HCM cases if they had not undergone familial genetic evaluation. A feature of *MYH7*-related disease is that there are major allelic differences in disease expression but the heterogeneity of disease manifestation within families is less marked. For example, it is unusual to encounter an Arg403Gln family with members who are non-penetrant; conversely Phe513Cys families do not have individuals with severe disease expression or major complications.[23,24,57]

Troponin T

Disease caused by *TNNT2* mutations is less common (3–5%) but potentially more dangerous than that caused by mutations in other sarcomeric genes. Systematic analysis of echocardiograms and outcomes in patients with *MYH7* and *TNNT2* mutations revealed less severe maximum wall thickness (mean thickness 22 mm vs 16 mm, respectively) but a higher incidence of premature sudden death in those with *TNNT2* mutations than in those with *MYH7* mutations.[30–32] Furthermore, a higher proportion of *TNNT2* patients who died suddenly had mild or absent LVH than did *MYH7* patients, a feature that has not been reported for any of the other HCM disease-causing genes. In patients who died suddenly, comparison of morphologic and histologic features of HCM patients with *TNNT2* mutations with those of HCM patients without *TNNT2* mutations showed that those with the *TNNT2* mutations were younger, had less hypertrophy and less fibrosis, but had more myocardial disarray than those without *TNNT2* mutations.[32] This particular mutation could represent the pathological substrate for malignant arrhythmias. Individuals with *TNNT2* mutations who have severe LVH have also been reported, but hypertrophy is usually only mild to moderate (13–20 mm). What is noteworthy, however, is that sudden death associated with

Table 1 Hypertrophic cardiomyopathy: disease-causing genes and associated phenotypes.

Gene	Protein	Frequency in patients with HCM	Associated phenotype
MYH7	β-Myosin heavy chain	25–35%	Mild or severe HCM
MYBPC3	Myosin-binding protein C (cardiac type)	20–30%	Expression similar to MYH7, late onset
TNNT2	Troponin T (cardiac muscle)	3–5%	Mild hypertrophy, sudden death
TNNI3	Troponin I (cardiac muscle)	<5%	Extreme intrafamilial heterogeneity, no sudden death without severe disease
TPM1	Tropomyosin 1α	<5%	Variable prognosis, sudden death
MYL2	Regulatory myosin light chain 2 (ventricular/cardiac-muscle isoform)	<5%	Skeletal myopathy
MYL3	Essential myosin light chain 3	Rare	Skeletal myopathy
ACTC	α-Cardiac actin 1	Rare	Apical hypertrophy
TTN	Titin	Rare	Typical HCM
TNNC1	Troponin C, slow skeletal and cardiac muscles	Rare	Typical HCM
MYH6	α-Myosin heavy chain	Rare	Late onset
CSRP3	Muscle LIM protein	Rare	Late onset, variable penetrance
MYLK2	Myosin light chain kinase 2	Rare	Early onset
LDB3	LIM binding domain 3	Rare	Mainly sigmoidal HCM
TCAP	Telethonin	Rare	Typical HCM, variable penetrance
VCL	Vinculin/metavinculin	Rare	Obstructive midventricular hypertrophy
ACTN2	α-Actinin 2	Rare	Mainly sigmoidal HCM
PLN	Phospholamban	Rare	Typical HCM, variable penetrance
MYOZ2	Myozenin 2	Rare	Typical HCM
JPH2	Junctophilin 2	Rare	Typical HCM

Abbreviation: HCM, hypertrophic cardiomyopathy.

TNNT2-related disease can occur in patients with mild or no clinical evidence of LVH.[31,60] The sudden death of a relative with HCM whose heart weight is normal or near normal should raise suspicion of *TNNT2* mutations.

Myosin-binding protein C

Clinical disease expression in individuals with HCM is usually progressive, beginning during childhood and adolescence in association with somatic growth, and completing by early adult years. Initial reports of *MYBPC3*-related disease revealed that onset of the disease phenotype occurs largely in the middle decades and that the mutation is associated with good prognosis.[27,28] These reports were confirmed by the finding that elderly patients with HCM often had *MYBPC3* mutations.[29] Since publication of these findings, however, further studies have described children with *MYBPC3* mutations who have severe disease phenotypes, indicating that the age of disease onset, severity of disease expression and risk of complications in these patients are similar to those of individuals with *MYH7*-related disease, with the proviso that late-onset disease can occur, albeit uncommonly, with *MYBPC3* mutations.[39]

Investigators in the Netherlands and South Africa have identified several *MYBPC3* mutations that exhibit founder effects and account for at least 30% of cases of HCM in their respective populations.[61,62] These mutations have relatively homogeneous clinical expression with mild to moderate hypertrophy and good prognosis.

Troponin I

TNNI3 mutations are associated with very heterogeneous disease expression. In one example, disease in three generations of patients comprised

Table 2 Hypertrophic cardiomyopathy phenocopies: disease-causing genes and associated phenotypes.

Gene	Protein	Frequency in patients with hypertrophic cardiomyopathy phenocopy diseases	Associated phenotype
PRKAG2	AMP-activated protein kinase γ^2 regulatory subunit	<1%	Wolff–Parkinson–White syndrome, conduction disease
GLA	α-Galactosidase A	<5%	Anderson–Fabry disease
GAA	Acid α-1,4-glucosidase	Rare	Pompe disease
AGL	Amylo-1,6-glucosidase	Rare	Forbes disease
LAMP2	Lysosome-associated membrane protein 2	Rare	Danon disease
Various mitochondrial genes (e.g. *MTTG*, *MTTI*)	Protein-coding mitochondrial ribosomal and transfer RNA	Rare	Mitochondrial cytopathy (MELAS, MERRF, LHON)
PTPN11	Tyrosine phosphatase SHP-2	Rare	LEOPARD syndrome, Noonan syndrome
FRDA	Frataxin	Rare	Friedreich's ataxia

Abbreviations: LHON, Leber's hereditary optic neuropathy; MELAS, mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes; MERRF, myoclonus epilepsy associated with ragged-red fibres.

mild asymmetric septal hypertrophy (grandmother), no disease features (mutation carrier, daughter) and 4-cm concentric hypertrophy (12-year-old grandson).[63] Symptoms can include severe restrictive physiology, severe biventricular hypertrophy and marked apical hypertrophy. There are families with major disease-related complications, as well as those with more mild forms of the disease (Figure 2).[63] Importantly, in contrast with *TNNT2*-related HCM, there have been no reports of sudden death in individuals with mild *TNNI3*-related disease.[63]

Other HCM-causing genes and compound mutations

Severe phenotypes have been reported in families with mutations in HCM-causing genes other than *MYH7*, *MYBPC3*, *TNNT2* and *TNNI3*. Unusual patterns of hypertrophy have been observed in patients harboring mutations in *ACTC* (α-cardiac actin 1; causes midcavity obstruction), and in *MYL2* and *MYL3* (regulatory and essential myosin light chains 2 and 3; cause midcavity obstruction and skeletal myopathy).[34,37]

Finding individuals in whom disease expression is severe and out of proportion to that observed in family members can indicate, at least in some cases, the presence of compound or multiple mutations. Compound mutations have been found in isolated families with *MYBPC3*-related HCM.[39] Approximately 3–5% of HCM patients with mutations in sarcomeric genes are heterozygous for two disease-causing mutations, either in the same or in different genes (i.e. compound or double heterozygotes).[14,39] As expected, these patients, as well as homozygous mutation patients, exhibit a more severe phenotype (i.e. higher penetrance, greater degree of hypertrophy and raised incidence of sudden death) than HCM cases with one causative mutation only.[64,65] This finding suggests that, in some cases, a gene–dosage effect might be responsible for the heterogeneous manifestations of the disease among family members.

Phenotypic variability

HCM exhibits variability in clinical expression in patients carrying the same disease-causing mutation. The extent of phenotypic heterogeneity between individuals with the same mutation differs from gene to gene and seems greatest with *TNNI3*. Phenotypic heterogeneity is seen in sarcomeric HCM as well as in phenocopies. This variability cannot be explained solely by the genetic defects involved or the type of mutation found. Disease manifestation is likely to be influenced by sex, genetic factors such as mutations, polymorphisms and modifier genes, and environmental factors such as lifestyle, degree of physical exercise and blood pressure.

Mutations in a variety of sarcomeric genes lead to pleiotropic cardiac phenotypes ranging from HCM to dilated cardiomyopathy;[38,66] each *TNNI3* mutation can be associated with diverse morphologies, and with both hypertrophic and restrictive cardiomyopathy, within the same family.[63]

Sex

Sex has been shown to influence disease progression in experimental models and in humans. In a transgenic mouse model of HCM (α-myosin heavy chain Arg403Gln mutation with a deletion in the actin-binding domain), males developed heart failure, whereas females did not.[67] Interestingly, early textbooks describe HCM as a disease of young men and older women. More recently, a study of 969 patients with HCM revealed that females were under-diagnosed and were older than men at the time of diagnosis.[68] In general, women with HCM had smaller ventricles and a higher proportion of women than men had left ventricular outflow obstruction. Women were also at increased risk of progression to NYHA functional class III or IV or of death from advancing heart failure or stroke, particularly if they were older than 50 years and had left ventricular outflow obstruction. The risk of sudden death, however, was similar in males and females.[68] By contrast, in athletes HCM-related sudden death appears to occur almost exclusively in males. In a previous report, 90% of 134 athletes with cardiovascular causes of sudden death were male.[6]

Polymorphisms of the renin–angiotensin–aldosterone system

The angiotensin-I converting enzyme (ACE) gene is a major component of the renin–angiotensin–aldosterone system (RAAS). The presence or absence of a 287-bp Alu repeat in intron 16 of this gene results in a common polymorphism that has three genotypes: II, ID and DD. These genotypes have been shown to influence the phenotypic expression of hypertrophy in patients with HCM.[69] In particular, the severity of LVH in individuals with HCM is greater in those carrying the DD polymorphism of the *ACE* gene than in those with DI or II polymorphisms.[69] This difference could relate to specific genes and mutations, as another study showed that the DD genotype was associated with LVH in patients with the *MYH7* Arg403Leu mutation, but not in those with other *MYH7* or *MYBPC3* mutations.[70]

A combined 'pro-LVH' polymorphism profile of five RAAS genes (*ACE*; angiotensinogen gene, *AGT*; angiotensin II receptor type 1 gene, *AGTR1*; aldosterone synthase gene, *CYP11B2*; and cardiac chymase A gene, *CMA*) was associated with the presence and degree of LVH in 26 family members who carried the *MYBPC3* mutation.[71] In another report, the degree of LVH in unrelated patients with mutations in *MYBPC3*, but not in

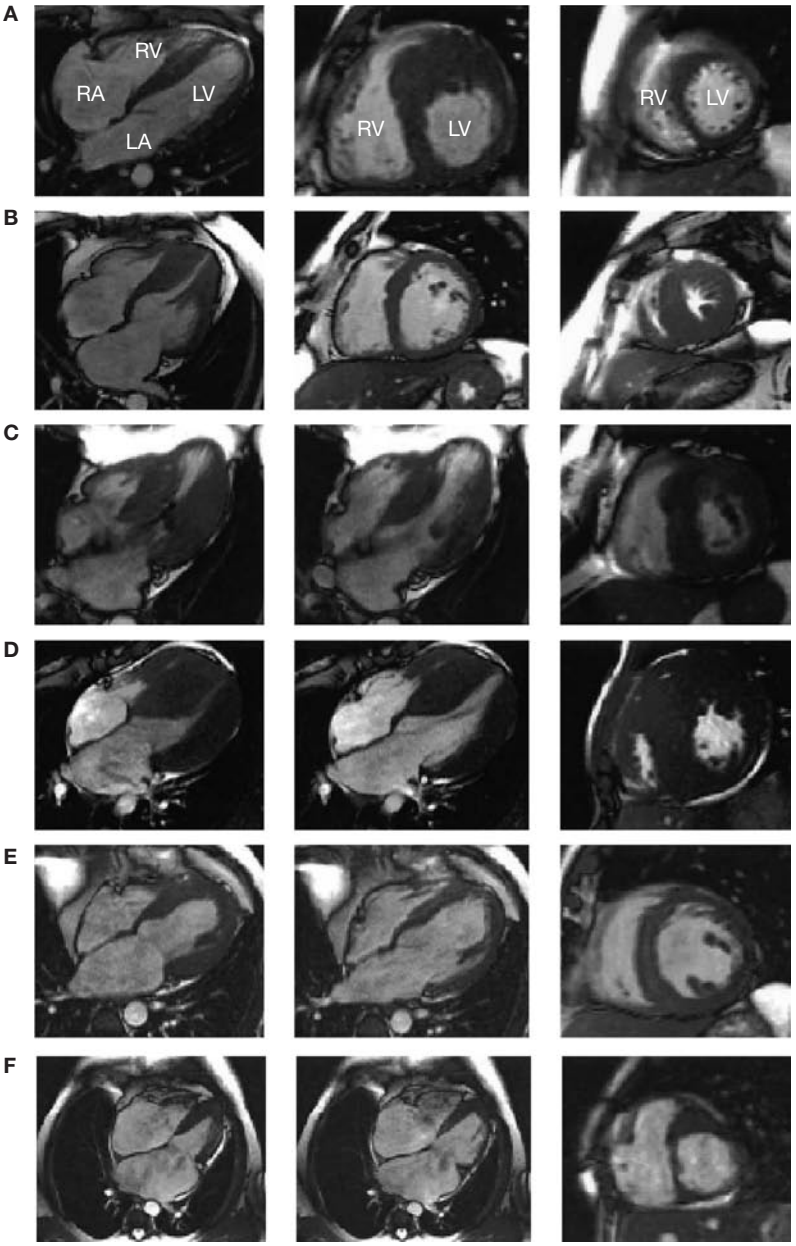

Figure 2 Cine cardiac MRI of hypertrophic cardiomyopathy patients with troponin I mutations. (**A**) Asymmetrical anteroseptal hypertrophy (Arg162Gln); (**B**) apical hypertrophy (Arg162Gln); (**C**) midcavity obstruction (Arg145Trp); (**D**) extreme biventricular hypertrophy (Arg141Gln); (**E**) 'end-stage' dilation (Arg186Gln); and (**F**) restrictive cardiomyopathy in a child 6 years of age (*de novo* Lys178Glu). (**A** and **B**) All images were captured during diastole; left-hand images show the four-chamber view, the central images show the cross-sectional view at the papillary muscle level, and the right-hand images show cross-sectional view at the apical level. (**C** to **F**) The left-hand images show the four-chamber view, captured during systole, the central images show the four-chamber view during diastole, and the right-hand images show the cross-sectional view at the papillary muscle level. Abbreviations: LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle. This figure was published in *Journal of the American College of Cardiology* Mogensen J *et al*. Frequency and clinical expression of cardiac troponin I mutations in 748 consecutive families with hypertrophic cardiomyopathy **44:** 2315–2325 © Elsevier (2004).

MYH7, correlated with five RAAS pro-LVH polymorphisms in the above mentioned genes.[72] These data suggest that individual genes of the RAAS have a limited influence on the degree of hypertrophy in HCM, but that their influence could be enhanced by synergistic pro-LVH polymorphisms of several genes.

Left ventricular remodeling

A minority (5–10%) of patients with HCM undergo unfavorable remodeling of the left ventricle with ventricular dilatation, wall thinning, decreased contractility, heart failure development and decreased survival.[73] This presentation is the strongest indication for heart transplantation in patients with HCM.[74,75] An increased familial tendency to develop left ventricular dysfunction and heart failure was found in 20% of the patients reported in one case series.[75] Specific mutations, such as *MYH7* Arg719Trp, tropomyosin 1α (*TPM1*) Glu180Val, and mutations in the α-myosin heavy chain gene (*MYH6*), were reported to be associated with a particularly high risk of cardiac dilatation and heart failure.[38,76,77] This presentation of HCM, coined 'end-stage' or 'burned-out' HCM, can manifest as decreased left ventricular contractility without significant wall thinning or ventricular dilatation, in the presence of significant fibrosis, and can be visualized by cardiac MRI with delayed hyperenhancement.[75,77] Patients reaching this phase of the disease are at increased risk of SCD and death resulting from heart failure.[74,75]

GENETIC TESTING IN HYPERTROPHIC CARDIOMYOPATHY

Genetic testing for diagnosis

The clinical application of mutation analysis is technically possible, but has been hindered by logistics and high cost. Some research centers in some national healthcare systems (UK, the Netherlands) offer testing on a selected basis, and it is commercially available via several private companies. Comprehensive mutation analysis remains the goal for all index cases with HCM. Given the cost of mutation analysis, however, a strategic approach based on probabilities should be employed where possible.

Careful phenotyping should identify the most common phenocopies of HCM. Glycogen storage disease can present with pre-excitation, premature conduction disease or skeletal myopathy. Mitochondrial disease is typically multiorgan and can involve vision and hearing systems and skeletal muscle. Exercise limitation that is disproportionate to hemodynamic and morphological severity of LVH often indicates skeletal muscle involvement and leads to correct phenocopy diagnosis. Noonan syndrome is also multiorgan and has a typical body habitus, which, although subtle, is highly characteristic. In general, young patients with Fabry's disease present with acroparasthesia and with pain on exercise or fever, while middle aged and older patients have angiokeratomata in the bathing trunk distribution, tinnitus and deafness, and renal impairment. Patients can occasionally present with unexplained stroke in the posterior cerebral circulation. Isolated cardiac symptoms can reflect Friedreich's ataxia (prior to development of the ataxia) or cardiac-variant Fabry's disease, but more often is due to a sarcomeric gene mutation. Strategies for performing mutation analysis are, on occasion, based on the cardiac phenotype; patients with late-onset disease, for example, are screened for *MYBPC3* mutations, or families with multiple sudden deaths and mild hypertrophy are tested for *TNNT2* mutations. More often than not, however, testing relies on disease probability on the basis of prevalence.

Some evidence suggests a correlation between the morphological shape of the cardiac septum and the presence of sarcomeric gene mutations. In particular, Binder *et al.* recently reported in a series of 382 patients with HCM that septal morphological subtype strongly predicted the presence or absence of such mutations.[78] This technique has not yet, however, been applied to patient screening.

As we highlighted previously, approximately 60% of cases of HCM are caused by mutations in sarcomeric genes. Systematic genotyping of nine sarcomeric genes (*MYH7, MYBPC3*, *TNNT2*, *TNNI3*, *TPM1* [tropomyosin 1α], *MYL2*, *MYL3*, *ACTC* and *TNNC1* [cardiac troponin C]) has revealed that more than 80% of disease-causing mutations are in *MYH7*, *MYBPC3* and *TNNT2.*[14] Genetic testing in an index case usually starts, therefore, with mutation analysis in these three most frequently affected genes, followed by analysis of the other sarcomeric genes (Figure 3). As there have been reports of patients with HCM carrying two mutations in sarcomeric genes,[14,39] it is recommended that screening of these nine genes is completed even when a mutation has already been detected. In the absence of finding a mutation in sarcomeric genes, it is advisable to reconsider possible HCM phenocopies such

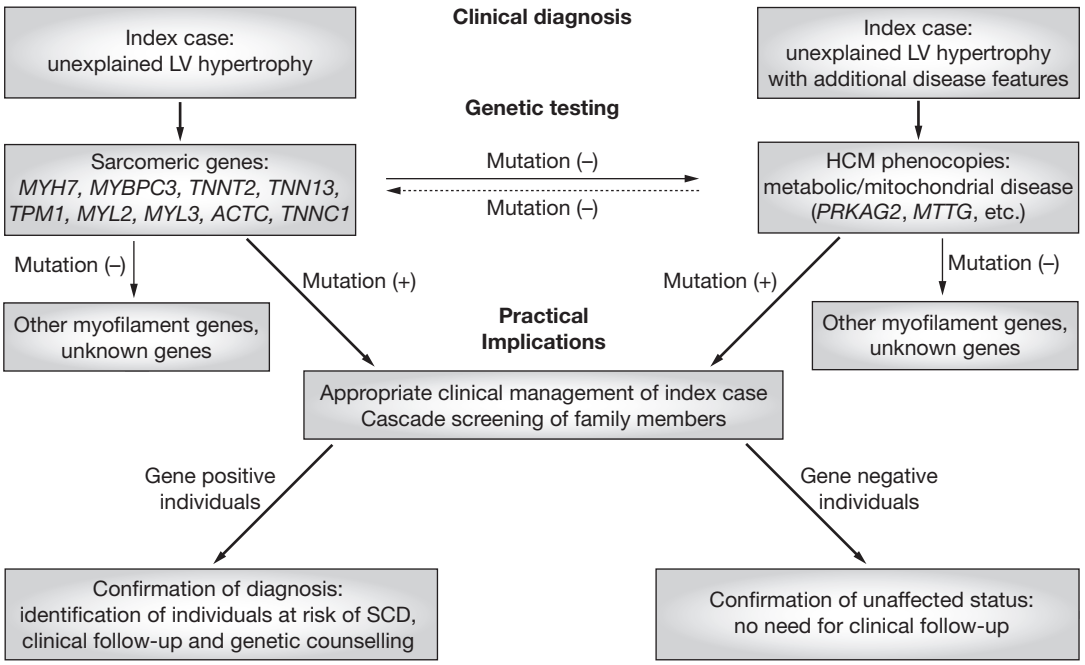

Figure 3 Proposed sequence of genetic testing for patients with hypertrophic cardiomyopathy. In order to detect double or compound heterozygotes complete screening of the nine most frequently mutated sarcomeric genes is recommended in the following order: *MYH7*, *MYBPC3*, *TNNT2*, *TNNI3*, *TPM1*, *MYL2*, *MYL3*, *ACTC* and *TNNC1*. Abbreviations: *ACTC*, α-cardiac actin; LV, left ventricular; *MTTG*, mitochondrially encoded tRNA glycine; *MYBPC3*, myosin-binding protein C; *MYH7*, β-myosin heavy chain; *MYL2*, regulatory myosin light chain 2; *MYL3*, essential myosin light chain 3; *PRKAG2*, AMP-activated protein kinase γ^2 regulatory subunit; *TNNI3*, troponin I; *TNNT2*, troponin T; *TPM1*, tropomyosin 1α; SCD, sudden cardiac death.

as Anderson–Fabry disease or Danon disease (Table 1); if these conditions are excluded from the diagnosis, additional screening of rare gene mutations should be considered. When a novel sequence change is found in one of the sarcomeric genes, determining whether it is a rare polymorphism or a disease-causing mutation is important; however, this distinction might prove particularly difficult for some missense mutations in *MYBPC3*[17] due to number of reasons such as insufficient data from functional studies, lack of genetic segregation in some families with reported mutations, and the fact that skeletal muscle biopsies are not useful as MYBPC3 protein is only expressed in the cardiac muscle. As a result, some sequence changes in *MYBPC3* are believed to be modifiers of cardiac hypertrophy rather than disease-causing mutations. In unusual presentations of HCM, such as extreme hypertrophy in infancy in the setting of familial disease with late presentation in other family members, searching for compound or double mutations is indicated.[14,17,18,39]

DNA analysis to identify mutations in genes associated with the disease is undoubtedly the most accurate method for establishing a diagnosis of HCM. Recently, Priori and Napolitano presented a novel scoring system to compare the value of genetic analysis in terms of cost: benefit ratio in different cardiomyopathies and ion channel diseases.[79] Their system is based on a number of technical and clinical criteria, such as size of genes to be screened, success rate of genotyping, and the clinical benefits patients and their relatives derive from the identification of a mutation. According to this system, genetic testing is indicated in diseases achieving a score of at least 3, whilst a score of 1 or less indicates that genotyping should be performed for research purposes only, as identification of the underlying mutation has limited clinical application. HCM falls into the high-score category, which highlights the suitability of genetic analysis for diagnosis of this disorder.[79]

Genetic testing for clinical management and prognosis

Genetic testing in patients with HCM provides prognostic and diagnostic benefits and can

substantially assist the clinical management of index cases and family members. Detection of a mutation in an index case increases the probability of diagnosis of uncertain cases in the same family who have nondiagnostic clinical features. Genetic analysis has prognostic value in certain index cases—*TNNT2* mutations, for example, are associated with increased risk of sudden death—but generally there are significant obstacles to accurate genetic prognostication. These obstacles include the existence of many 'private' mutations with unknown functional characteristics, and variable disease severity, seen even among patients carrying identical mutations.

Arguably, an important benefit of genetic analysis is the potential of preclinical diagnosis in patients with a family history of SCD or in those carrying a 'malignant' mutation that predisposes them to a severe phenotype. Moreover, an advantage of knowing the type of genetic defect in HCM is the possibility of identifying familial asymptomatic mutation carriers who might be at risk of SCD.

Once a mutation has been detected in a proband, the possibility of genetic testing should be suggested to first-degree relatives, who have a 50% probability of being gene-positive ('cascade' screening). This type of screening enables close clinical management of mutation carriers, and identifies genotypically normal family members, obviating the need for them to undergo clinical screening and repeat follow-up examinations.

Implications of genetic testing and counseling

Information obtained by DNA analysis should be conveyed to the family in question through detailed genetic counseling. This process should include analysis of the genetic implications of the disease and discussion about available therapies, as well as advice on how the diagnosis will affect the patient's daily routine, leisure activities, career, medical insurance and family planning.

Even though in some cases the benefits gained from identifying a pathogenic mutation in a proband can be substantial, in others the same discovery could have important negative effects. In particular, detecting mutations associated with increased risk of sudden death, for example *TNNT2* mutations, or bad prognosis, for example Arg403Gln and Arg453Cys mutations in *MYH7*, can present a family with complex and difficult issues to consider. These issues could range from medical (i.e. clinical management), legal (i.e. insurance) and social (i.e. lifestyle, family planning, stigmatization), to psychological (i.e. feelings of anxiety or guilt). Genetic testing might not, therefore, be appropriate for every case of HCM, and a decision whether to offer it should be made within the context of and with the cooperation of each particular family. Such considerations would consequently preclude a blanket application of DNA testing for HCM.

CONCLUSIONS

HCM is a common inherited cardiac disease with remarkable clinical and genetic heterogeneity. Mutations in genes encoding proteins of the sarcomere are responsible for the disease in most cases, but mutations in other genes have also been implicated. A large proportion of patients with unexplained LVH (~40%) have no mutations in sarcomeric genes, highlighting the presence of phenocopies. These similar conditions include various mitochondrial and metabolic diseases that phenotypically overlap with pure HCM. Molecular genetic testing can benefit patients by facilitating accurate diagnosis and identifying gene-positive or gene-negative individuals; however, its use should be considered on an individual basis within the context of each family. Used appropriately, genetic analysis can have an important role in the prognosis, genetic counseling and clinical management of patients with HCM and their families.

KEY POINTS

- Hypertrophic cardiomyopathy (HCM) is a common inherited cardiac disorder that is defined clinically by the presence of unexplained left ventricular hypertrophy
- HCM is a major cause of sudden cardiac death, especially in the young and in seemingly healthy athletes
- Mutations in genes encoding sarcomeric proteins are detected in 60% of patients with HCM
- Several diseases mimic the phenotypic expression of HCM (phenocopies); it is clinically important to recognize phenocopies as the genetics, clinical management and prognosis of these conditions differ to sarcomeric HCM
- Used appropriately, genetic testing can be important in the prognosis, clinical management and genetic counseling of patients and their families

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