

FOCUS ISSUE: HYPERTROPHIC CARDIOMYOPATHY

Diagnostic, Prognostic, and Therapeutic Implications of Genetic Testing for Hypertrophic Cardiomyopathy

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Over the last 2 decades, the pathogenic basis for the most common heritable cardiovascular disease, hypertrophic cardiomyopathy (HCM), has been investigated extensively. Affecting approximately 1 in 500 individuals, HCM is the most common cause of sudden death in young athletes. In recent years, genomic medicine has been moving from the bench to the bedside throughout all medical disciplines including cardiology. Now, genomic medicine has entered clinical practice as it pertains to the evaluation and management of patients with HCM. The continuous research and discoveries of new HCM susceptibility genes, the growing amount of data from genotype-phenotype correlation studies, and the introduction of commercially available genetic tests for HCM make it essential that the modern-day cardiologist understand the diagnostic, prognostic, and therapeutic implications of HCM genetic testing. (J Am Coll Cardiol 2009;54:201-11) © 2009 by the American College of Cardiology Foundation

Affecting 1 in 500 people, hypertrophic cardiomyopathy (HCM) is a disease marked by phenotypic and genotypic heterogeneity and is the most prevalent, heritable cardiovascular disease. HCM is the most common cause of sudden cardiac death in young athletes (1). HCM can manifest negligible to extreme hypertrophy, minimal to extensive fibrosis and myocyte disarray on microscopy, absent to severe left ventricular (LV) outflow tract obstruction, and distinct septal morphologies such as reverse curve, sigmoidal, and apical HCM. The clinical course varies extremely as well, ranging from an asymptomatic lifelong course to dyspnea and/or angina refractory to pharmacotherapy to sudden death as the sentinel event. Fully described for the first time by Teare (2) in 1958, HCM was regarded as “asymmetrical hypertrophy of the heart in young adults.” It has since been referred to by an array of names—idiopathic hypertrophic subaortic stenosis (3), muscular subaortic stenosis (4), and hypertrophic obstructive cardiomyopathy (5)—reflecting its clinical heterogeneity and its relatively uncommon occurrence in daily cardiologic practice.

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Diagnostic Implications of HCM Genetic Testing

Identification of HCM susceptibility genes. Nearly 20 years ago, the first chromosome locus for familial HCM and subsequently mutations involving the *MYH7*-encoded beta-myosin heavy chain were elucidated as the pathogenic basis for HCM (6,7). Since then several hundreds of mutations scattered among at least 27 putative HCM susceptibility genes encoding various sarcomeric, calcium-handling, and mitochondrial proteins have been identified (Tables 1 and 2). The most common genetically mediated form of HCM is myofilament (sarcomeric)-HCM with hundreds of disease-associated mutations in 9 genes encoding proteins (myofilaments) critical to the cardiac sarcomere. This includes beta-myosin heavy chain (*MYH7*) (7), regulatory (*MYL2*) and essential myosin light chains (*MYL3*) (8), myosin binding protein C (*MYBPC3*) (9), cardiac troponin T (*TNNT2*), alpha-tropomyosin (*TPM1*) (10), cardiac troponin I (*TNNI3*) (11), cardiac troponin C (*TNNC1*) (12), and actin (*ACTC*) (13,14). Complete screening through a large cohort of patients has not been performed, yet targeted screening of giant sarcomeric *TTN*-encoded titin, which extends throughout one-half of the sarcomere, has thus far revealed only 1 mutation (15).

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 1). Due to its close proximity to the contractile apparatus of the myofilament, its specific structure-function relationship with regard to cyto-

Abbreviations and Acronyms

- ACE** = angiotensinogen-I converting enzyme
- DCM** = dilated cardiomyopathy
- HCM** = hypertrophic cardiomyopathy
- LV** = left ventricle/ventricular
- LVH** = left ventricular hypertrophy
- LVNC** = left ventricular noncompaction
- miRNA** = micro-ribonucleic acid
- RAAS** = renin-angiotensin-aldosterone system
- RNA** = ribonucleic acid

architecture, 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 fueled by the fact 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 (16–24). The first Z-disc mutations associated with HCM were described in muscle LIM protein encoded by *CSR3* (21) and telethonin encoded by *TCAP* (23). Recently, *LDB3*-encoded LIM domain binding 3, *ACTN2*-encoded alpha actinin 2, *VCL*-encoded vinculin/metavinculin (24–26), and *MYOZ2*-encoded myozenin-2 (27) have been added to that list. In another demonstration of mutations in 1 gene causing multiple diseases, *MYPN*-encoded myopalladin (*MYPN*) mutations were implicated in the pathogenesis of DCM, HCM, and restrictive cardiomyopathy 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 hy-

pothesis 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 (28,29). 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, may cause HCM (30).

New insights and approaches to genomics of cardiac hypertrophy and HCM. Not only in molecular genetics but also in other fields of ‘-omics,’ novel pathways underlying the pathophysiology of cardiac hypertrophy and HCM have been identified using several new techniques to study large-scale transcriptional changes (31–33). A transcriptomic approach can be performed by using microarray, a technique that enables a snapshot view of gene expression, which, combined with complex analytic tools, can identify genes that are differentially regulated or seem to be coregulated, thereby forming a transcriptional network of genes and pathways. Microarray chips can hold over tens of thousands of genes and can be utilized to compare expression levels in certain disease states with healthy control subjects.

In 2002, Hwang et al. (34) studied ribonucleic acid (RNA) from heart failure patients with either HCM or DCM and found almost 200 genes to be up-regulated and 51 genes down-regulated in both conditions, as well as several genes differentially expressed between the 2 diseases

Table 1 Summary of Hypertrophic Cardiomyopathy Susceptibility Genes

Gene	Locus	Protein	Frequency (%)
Myofilament HCM			
<i>TTN</i>	2q24.3	Titin	<1
<i>MYH7</i>	14q11.2–q12	Beta-myosin heavy chain	15–25
<i>MYH6</i>	14q11.2–q12	Alpha-myosin heavy chain	<1
<i>MYL2</i>	12q23–q24.3	Ventricular regulatory myosin light chain	<2
<i>MYL3</i>	3p21.2–p21.3	Ventricular essential myosin light chain	<1
<i>MYBPC3</i>	11p11.2	Cardiac myosin-binding protein C	15–25
<i>TNNT2</i>	1q32	Cardiac troponin T	<5
<i>TNNI3</i>	19p13.4	Cardiac troponin I	<5
<i>TPM1</i>	15q22.1	Alpha-tropomyosin	<5
<i>ACTC</i>	15q14	Alpha-cardiac actin	<1
<i>TNNC1</i>	3p21.3–p14.3	Cardiac troponin C	<1
Z-disc HCM			
<i>LDB3</i>	10q22.2–q23.3	LIM binding domain 3 (alias: ZASP)	1–5
<i>CSR3</i>	11p15.1	Muscle LIM protein	<1
<i>TCAP</i>	17q12–q21.1	Telethonin	<1
<i>VCL</i>	10q22.1–q23	Vinculin/metavinculin	<1
<i>ACTN2</i>	1q42–q43	Alpha-actinin 2	<1
<i>MYOZ2</i>	4q26–q27	Myozenin 2	<1
Calcium-handling HCM			
<i>JPH2</i>	20q12	Junctophilin-2	<1
<i>PLN</i>	6q22.1	Phospholamban	<1

Genes in bold are available as commercial genetic tests.

Gene	Locus	Protein	Syndrome
TAZ	Xq28	Tafazzin (G4.5)	Barth syndrome/LVNC
DTNA	18q12	Alpha-dystrobrevin	Barth syndrome/LVNC
PRKAG2	7q35–q36.36	AMP-activated protein kinase	WPW/HCM
LAMP2	Xq24	Lysosome-associated membrane protein 2	Danon's syndrome/WPW
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
KRAS	12p12.1	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog	Noonan's syndrome
SOS1	2p22-p21	Son of sevenless homolog 1	Noonan's syndrome

Genes in bold are available as commercial genetic tests.

AMP = adenosine monophosphate; HCM = hypertrophic cardiomyopathy; LEOPARD = mnemonic for syndrome with clinical characteristics of lentiginosities, electrocardiographic conduction abnormalities, ocular hypertelorism, pulmonary hypertension, abnormal genitalia, retarded growth, deafness; LVNC = left ventricular noncompaction; WPW = Wolff-Parkinson-White syndrome.

providing information on different pathways and genes involved in the pathogenesis. Rajan et al. (35) performed microarray analysis on ventricular tissue of 2 previously developed transgenic, 2.5-month-old HCM mice (alpha-TM175 and -TM180) carrying mutations in alpha-tropomyosin (*TPM1*). Studying 22,600 genes, 754 differentially expressed genes between transgenic and nontransgenic mice were detected, of which 266 were differentially regulated between the 2 different mutant hearts showing most significant changes in genes belonging to the 'secreted/extracellular matrix' (up-regulation) and 'metabolic enzymes' (down-regulation).

Another emerging field is that of micro-ribonucleic acids (miRNAs) and their role in cardiac development and (hypertrophic) heart disease. These fundamental cellular regulators were first described by Lee et al. (36) in 1993 and consist of approximately 22 noncoding ribonucleic acid molecules that silence genes through post-transcriptional regulation. miRNAs play an important role in cardiac development as well as in orchestrating organogenesis and early embryonic patterning processes (37,38). Furthermore, these noncoding ribonucleic acid molecules seem to play an important role in cardiac remodeling and the development of hypertrophy as initially reported by van Rooij et al. (39) in 2006. Utilizing 2 mouse models of pathological hypertrophy, transverse aortic constriction, and calcineurin transgenic mice, 6 miRNAs were up-regulated, which in vitro were sufficient to induce hypertrophic growth of cardiomyocytes (39). Furthermore, a transgenic mouse model overexpressing 1 of these miRNAs (miR-195) showed that a single miRNA could induce pathological hypertrophy and heart failure (39). Over the last year, multiple studies have been published with miRNA expression profiles in different settings, in vivo and in vitro, of cardiac hypertrophy (37,39,40–43). Lastly, polony multiplex analysis of gene expression (PMAGE) is a technique

that detects messenger RNAs as rare as 1 transcript per 3 cells (44). Using this new technique, early transcriptional changes preceding pathological manifestations were identified in mice with HCM-causing mutations, including low-abundance messenger RNA encoding signaling molecules and transcription factors that participate in the disease pathogenesis (44).

The development and implementation of these new techniques as well as their applications in research and clinical models of cardiac hypertrophy and HCM will over the years teach us more about the pathophysiology of normal and pathological hypertrophy as well as HCM. This, in turn, might lead to discovery of novel disease-causing genes, involved pathways, and possible novel therapeutic targets.

HCM genetic testing in clinical practice. Recently, HCM genetic testing has matured from its 2-decade long residence in research laboratories into the realm of clinically available, diagnostic testing for physicians evaluating and treating patients with this disease (Harvard Partners, Correlagen, PGxHealth, and GeneDX). These companies now offer testing for the 8 most common myofilament associated genes; additional genes offered by some are the genes involved in the glycogen storage diseases or the recently discovered HCM-associated gene troponin-C encoded by *TNNC1*. The HCM susceptibility genes available for commercial genetic testing are highlighted in bold in Tables 1 and 2.

Although some of the new HCM susceptibility genes may surpass the prevalence of mutations found in some of the myofilament proteins, *MYBPC3* and *MYH7* remain by far the most common HCM-associated genes, with an estimated prevalence of 15% to 25% for both genes. Among the 9 HCM-associated, myofilament-encoding genes, the prevalence of myofilament HCM has ranged from 35% to

65% in several different international cohorts of unrelated patients who met the clinically accepted definition of HCM (45,46).

Echocardiography-guided genetic testing. While several phenotype-genotype relationships have emerged to enrich the yield of genetic testing, most of these patient profiles have not been particularly clinically informative. Recently, the possibility of echocardiography-guided genetic testing has been explored (47). Noting a predominance of sigmoidal HCM among the elderly, Lever et al. (48) suggested over 2 decades ago that there was a strong age dependence with the various septal morphologies of HCM, where septal contour was classified as reverse curve, sigmoidal, apical, and neutral contour HCM (Fig. 1). In the early 1990s, Solomon et al. (49) observed that patients with mutations in the beta-myosin heavy chain (MYH7-HCM) generally had reversed curvature septal contours (reverse curve HCM).

Subsequently, a large genotype-phenotype analysis correlating the septal morphology with the underlying genotype was conducted. After extensive analysis of the echocardiograms of nearly 400 unrelated patients, sigmoidal HCM (47% of cohort) and reverse curve HCM (35% of cohort) represented the 2 most prevalent anatomical subtypes of HCM (Fig. 1). In this study, the yield of genetic testing for myofilament HCM (8 genes) was 80% in reverse curve HCM, but only 10% in patients with sigmoidal HCM, and septal contour was the strongest predictor of a positive HCM genetic test, regardless of age (odds ratio: 21, $p < 0.0001$) (47). These observations may facilitate echocardiography-guided genetic testing by enabling informed genetic counseling about the *pre-test* probability of a positive genetic test based upon the patient's expressed anatomical phenotype (Fig. 2).

Role of HCM genetic testing for both index cases and relatives. Although in the future there may be some prognostic relevance presently and therapeutic relevance to the HCM genetic test in the index cases that already

clinically manifest the disease, the principal role for index case genetic testing is diagnostic. It can, however, as we will show later on, be of significant importance to the approach and screening of relatives.

Figure 2 provides a flow chart for clinicians, in which the 2 pathways are described when an index case (HCM proband) is identified. It must be recognized that, before a diagnosis of HCM in the proband is made, a full history, examination, including extensive family history, should be performed. This way, clues can be picked up to expose other causes of unexplained left ventricular hypertrophy (LVH)—aortic stenosis, hypertension, or the presence of a phenocopy—as being responsible for the patient's symptoms. For example, signs of ventricular pre-excitation might point to a *PRKAG2*-mediated glycogen storage disease or an inheritance pattern that strictly affects male patients might suggest *LAMP2*-mediated disease. If the phenotype is HCM, echocardiography may inform genetic counseling by providing an a priori probability for a positive genetic test and advice on how to proceed with further evaluation and family screening (left arm of algorithm). If genetic testing of the major genes remains negative, the presence of a phenocopy with pure cardiac involvement should be considered.

As it stands now, genetic testing of the index case has the potential of providing the diagnostic gold standard for his/her offspring, siblings, and 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 risk 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 rendered, all first-degree

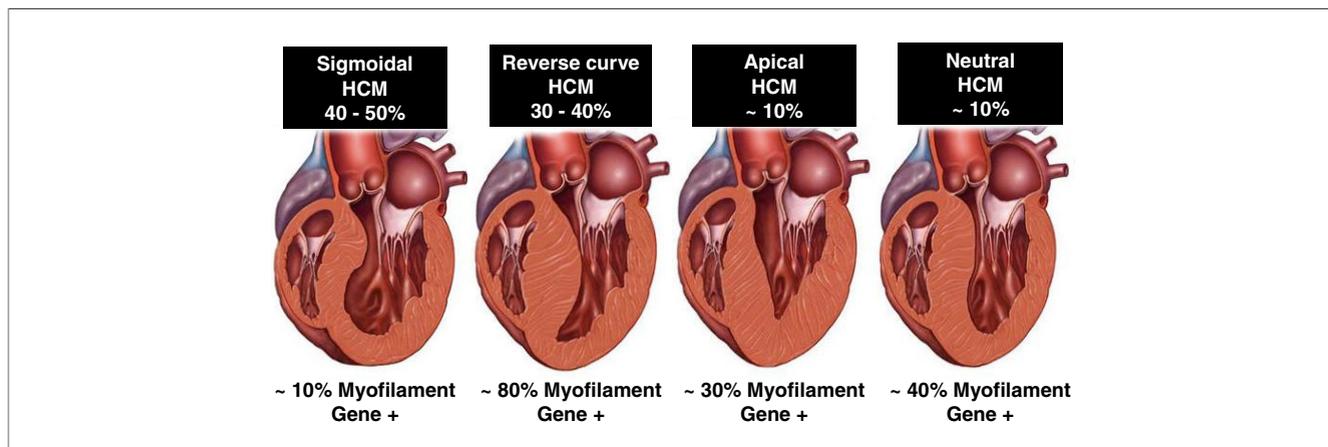


Figure 1. Septal Morphologies in HCM

Shown are the most common septal morphologies in hypertrophic cardiomyopathy (HCM). The distribution of septal morphologies among a large cohort of patients with HCM is shown along the **top** while the yield of genetic testing for each morphological subgroup is shown along the **bottom** of the figure.

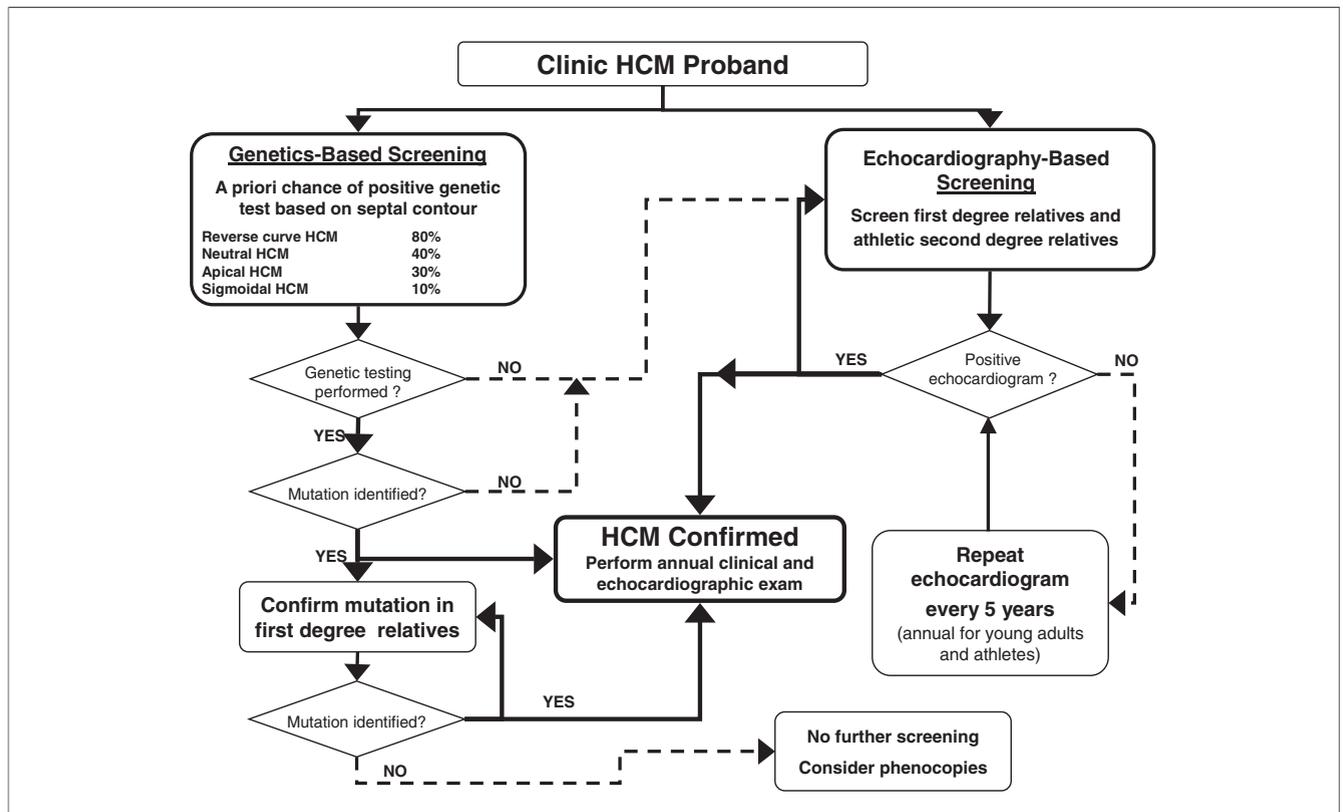


Figure 2 Genetic- and Echocardiographic-Based Screening in HCM

Flow chart showing a possible decision tree to follow in genetic- and echocardiography-based screening in hypertrophic cardiomyopathy (HCM). Noted is the a priori probability for a positive genetic test result based on the echocardiographic scored septal contour, as well as the steps to follow if a patient chooses not to pursue genetic testing.

relatives and probably 'athletic' second-degree relatives to the index case should be screened by an electrocardiogram and echocardiogram. Annual screenings are recommended for adolescents and young adults (age 12 to 25 years) and athletes, and thereafter every 3 to 5 years. As intimated previously, if an HCM-causing mutation is established for the index case, first-degree relatives should have confirmatory genetic testing performed for that particular HCM-causing mutation. Depending on the established familial versus sporadic pattern, confirmatory genetic testing should proceed in concentric circles of relatedness.

For example, if the index case's mutation is present in his/her father, then the paternal grandparents and paternal aunts/uncles should be tested. The index case's first-degree cousins may or may not need genetic testing depending on the results of the testing among the aunts and uncles and so forth. If a phenotype-negative family member tests *negative* for the index case's mutation, then future cardiologic evaluations for that relative and his/her progeny may not be necessary. However, a decision to cease surveillance for HCM in a relative hinges critically on the certainty of the identified gene/mutation and its causative link as well as the complete absence of any

traditional evidence used to clinically diagnose HCM (i.e., asymptomatic and normal echocardiogram).

Aside from the role of genetic testing described in the preceding text, there is still concern among patients and practitioners on social and economic aspects of knowing ones genetic make-up. For example, genetic testing may or may not be covered by an individual's health insurance plan. Some payors view HCM genetic testing as a bona fide clinical test while others view it as an 'investigational' one. Secondly, if genetic testing is performed, a patient might feel anxiety about the potential clinical dangers of hosting an HCM-predisposing mutation. Also, there might be fears that the presence of this information in one's medical records might influence health insurance premiums and employment opportunities, although this last issue has been addressed recently by former President Bush's signing of the Genetic Information Nondiscrimination Act (GINA) into law. This is an important step to provide protection against genetic discrimination. Besides discussing the differential impact in terms of follow-up for a genotype-positive relative compared with a genotype-negative relative as described earlier, these important issues should also comprise the genetic counseling.

Novel imaging techniques for early detection of HCM disease gene expression. With growing knowledge on genetics and other pathogenetic pathways in HCM, it would be very helpful if parameters could be found that suggest pre-clinical, pre-hypertrophic expression of the genetic substrate. Tissue Doppler echocardiography studies in transgenic rabbit models of HCM (50–52) and in humans have shown reduced myocardial Doppler velocities in genotype-positive subjects without LVH (50,53–55). Nagueh et al. provided the first evidence in 2001 demonstrating that myocardial contraction and relaxation velocities as detected by tissue Doppler are reduced in familial, mutation-positive HCM (54). In 2002, Ho et al. (55) showed that abnormalities of diastolic function as assessed by Doppler tissue imaging precede development of LVH in patients with *MYH7* mutations where a combination of Ea velocity and ejection fraction was highly predictive of affected phenotype in patients without hypertrophy (55). Cardiac magnetic resonance imaging is also showing potential to become an important tool in the diagnosis of HCM as it has capacity to acquire images with tissue contrast and border definition that is often superior to echocardiography (56–59). In 2006, Germans et al. (60) performed cardiac magnetic resonance imaging on 16 mutation-positive HCM patients and detected pre-hypertrophic crypts in the inferoseptal LV wall, possibly representing early pathological alterations stemming from the pathogenetic substrate.

Prognostic Implications of HCM Genetic Testing

From the early beginnings of the genomic era and since the description of the first HCM-causing mutation, investigators have attempted to correlate genotypes to particular clinical phenotypic expressions. Stemming from earlier pedigree studies, specific missense mutations were associated with a markedly unfavorable prognosis whereas others had an uneventful natural history. These observations resulted in specific mutations being designated as either ‘malignant’ mutations or ‘benign’ mutations (61–68). The first study of its kind was published by Watkins et al. (61) in 1992 in which they described mutations in *MYH7* found in 12 of 25 families with HCM. They concluded that the *MYH7*-R403Q mutation was associated with a significantly shorter life expectancy, and could therefore be considered a ‘malignant’ mutation. In contrast, a noncharge change mutation (V606M) was associated with nearly normal survival and therefore was considered ‘benign’ (61). In 2003, Woo et al. (69) analyzed mutations in functional domains in 15 (of 70) *MYH7*-positive probands and concluded that there may be prognostically informative domains.

Initial reports on the clinical expression from the most common subtype of HCM—*MYBPC*-HCM—seem to show a slower, but progressive clinical disease course, with later onset and milder disease characteristics (68,70,71). Investigators in the Netherlands and South Africa have discovered founder mutations in *MYBPC3* with mild phe-

notypic expression that are present in at least 30% of their cases (72). Similarly, certain genotype-phenotype correlations were attributed to *TNNT2* (troponin T)-HCM. Far less common than *MYBPC*-HCM or *MYH7*-HCM, *TNNT2*-HCM (affecting <5% of patients) was associated with less severe LV wall thickness, but a higher incidence of premature sudden cardiac death (64,73,74). Overall, these *TNNT2*-HCM patients who suddenly died had less hypertrophy and less fibrosis, but more myocyte disarray, which may have provided the substrate for malignant arrhythmias (74).

Overall, these observations have been gleaned from small cohorts involving larger families with penetrant disease expression whereas genotype-phenotype studies involving large cohorts of unrelated patients have indicated that great caution must be exercised with assigning particular prognostic significance to any particular mutation (75–77). In 1 such cohort, only 2% hosted 1 of those formally annotated ‘benign’ mutations and, moreover, these particular hosts displayed a severe clinical phenotype with all 5 patients requiring surgical myectomy, 3 of the 5 having a family history of sudden cardiac death, and 1 adolescent requiring an orthotopic heart transplant (77). In contrast, 3 patients hosting a so-called ‘malignant’ mutation displayed a heretofore mild phenotype (75). Furthermore, these studies have demonstrated that the 2 most common forms of genetically mediated *MYH7*-HCM and *MYBPC3*-HCM—are phenotypically indistinguishable (78).

More recently, in one of the first studies of its kind for HCM, a longitudinal study in a large cohort of unrelated Italian patients with HCM has shown an increased risk of cardiovascular death, nonfatal stroke, or progression to New York Heart Association functional class III/IV among patients with a positive HCM genetic test involving any of the myofilament genes compared with those patients with a negative genetic test (25% vs. 7%, respectively; $p = 0.002$) (Fig. 3A); multivariate analysis showed myofilament-positive HCM (i.e., a positive genetic test) to be the strongest predictor of an adverse outcome (hazard ratio: 4.27 [95% confidence interval: 1.43 to 12.48]; $p = 0.008$) (79). Furthermore, patients with myofilament genotype-positive HCM had greater probability of developing severe LV systolic dysfunction ($p = 0.021$) (Fig. 3B) and restrictive LV filling ($p = 0.018$) (Fig. 3C).

Lastly, it has been observed that patients with multiple mutations (i.e., compound or double heterozygotes), detected in about 3% to 5% of genotype-positive patients, have a more severe phenotype and increased incidence of sudden death (78,80,81), suggesting a gene-dosage effect might contribute to disease severity. Interestingly, in the majority of cases of compound heterozygosity, one of the mutations usually involves *MYBPC3* (78). In their longitudinal study, Olivetto et al. (79) observed a similar trend showing that patients with double mutations (of which 1 was usually *MYBPC3*) had greater disease severity than myofilament-negative patients or patients with a single *MYBPC3*, thick

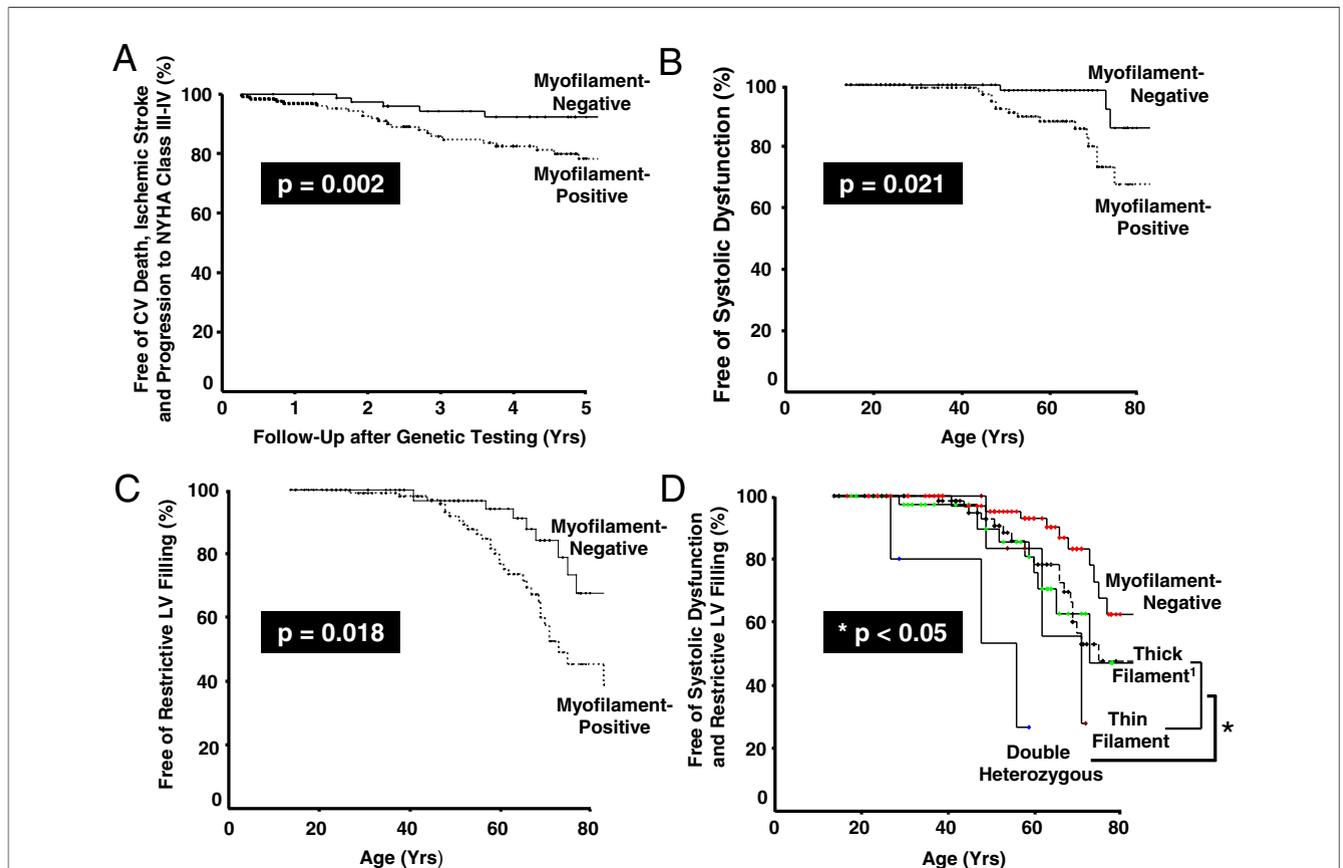


Figure 3 Relation of Genetic Test Status to Outcome in Patients With Hypertrophic Cardiomyopathy

Follow-up data show that patients harboring a myofilament mutation (i.e., a positive genetic test) progress to cardiovascular (CV) death, ischemic stroke, or New York Heart Association (NYHA) functional class III to IV more rapidly than patients with a negative genetic test (A). Furthermore, patients with a myofilament mutation are more likely to develop systolic dysfunction (B) or a restrictive filling pattern (C), independent of the genotype involved (D). 1) Includes mutations in β -myosin heavy chain (MYH7), myosin binding protein C (MYBPC3), and regulatory and essential myosin light chains (MYL2 and MYL3). LV = left ventricular.

filament, or thin filament mutation combined ($p < 0.05$) (Fig. 3D).

In summary, although clinical prognostication must be rendered with great caution for specific gene domains or specific genetic mutations, a positive HCM genetic test in general portends a greater likelihood for disease progression, particularly as it pertains to systolic and diastolic dysfunction and propensity to develop symptoms. As such, clinical genetic testing may thereby aid in the prognostication of a patient's disease outcome.

Interpretation of rare variants and phenocopies. One group of patients that pose an intriguing challenge for clinicians is that of patients with seemingly unexplained LVH that mimics the HCM phenotype. These diseases are usually referred to as phenocopies, and the most important ones are listed in Table 2. Phenocopies or rare variants pose a tough dilemma for the clinician. If the phenotype does not look like typical HCM and other symptoms like ventricular pre-excitation or muscle weakness 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.

A different cardiomyopathy and phenocopy that can present itself with seemingly unexplained hypertrophy is that of left ventricular noncompaction (LVNC)—a primary cardiomyopathy characterized by a severely thickened 2-layered myocardium, numerous prominent trabeculations, and deep intertrabecular recesses (82,83). Although genetically still largely unexplained, mutations in the *TAZ*-encoded tafazzin (G4.5)—also associated with Barth syndrome—*DTNA*-encoded alpha-dystrobrevin and *LDB3* have been associated in the pathogenesis of LVNC (84–86). Recently, Klaassen et al. (87) systematically analyzed a cohort of 63 unrelated patients with LVNC for mutations in 6 myofilament-encoding genes, identifying 9 distinct heterozygous mutations in 11 patients in MYH7, ACTC, and TNNT2 suggesting that there might be a shared etiology

for the myofibrillar forms of the common allelic cardiomyopathies of HCM, DCM, and LVNC.

Some diseases presenting chiefly with cardiac hypertrophy turn out to have clearly distinct underlying pathophysiologies. In 2001, 2 independent groups discovered *PRKAG2* mutations being involved in families with cardiac hypertrophy and ventricular pre-excitation, conduction abnormalities, and signs of Wolff-Parkinson-White syndrome (88–90). In 2005, Arad et al. (91) also described mutations in lysosome-associated membrane protein-2 encoded by *LAMP2* and *PRKAG2* and found that underlying glycogen storage diseases mimicked the clinical phenotype of HCM. Yang et al. (92) showed that *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.

Role of modifiers in HCM. The role of modifiers of the HCM phenotype, either by the presence of common polymorphisms or founder mutations, has become the subject of recent investigations. The most important subgroup of polymorphisms, studied to date, involve the major components of the renin-angiotensin-aldosterone system (RAAS). 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]: DD-*ACE*, CC-*AGTR1*, AA-*CMA*, T174M- and M235T-AGT, and CC-*CYP11B2*) appear to influence the HCM phenotype, in particular the severity of LVH (93,94). Among patients with the DD-*ACE* genotype, there was greater LVH than among those with an ID or II genotype (95). 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 (93) and in a large cohort of myofibrillar-positive patients (94).

In 2008, sex hormone polymorphisms were shown to modify the HCM phenotype (96). Fewer CAG-nucleotide repeats in *AR*-encoded androgen receptor were associated with thicker myocardial walls in male subjects ($p = 0.008$), 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 ($p = 0.047$) compared with GG-homozygote male subjects (96). HCM modifier polymorphisms like these could contribute to the clinical differences observed between men and women with HCM (97,98). 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. (99) 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 ~8 g shift from 1 locus for the common allele to 90 g shift for another locus' uncommon allele.

Therapeutic Implications of HCM Genetic Testing

Pharmacogenomics. Currently, there is no available therapy specifically designed to target specific HCM-causing gene mutations or particular HCM genotypes. Further, no therapies have been shown to reverse the hypertrophic process in humans. One of the first studies of its kind was performed in transgenic MYH7-R403Q mice models (designated $\alpha MHC^{403/+}$). In a randomized trial, $\alpha MHC^{403/+}$ mice treated with diltiazem, an L-type calcium inhibitor, showed significant improvement as compared with mice treated with placebo in terms of cardiac systolic function as measured by increased end-diastolic and -systolic volumes, decreased dP/dT_{max} values, and end-systolic elastance (100). Furthermore, diltiazem-treated mice showed significantly less hypertrophy at 30 and 39 weeks than age-matched $\alpha MHC^{403/+}$ untreated mice as well as less fibrosis and myocyte disarray on microscopy (100). Recently, Westermann et al. (101) showed in a different transgenic mouse model (TNNT2-I79N) that diltiazem improved diastolic function and prevented diastolic heart failure and sudden cardiac death compared with that seen in untreated mice.

As previously discussed, RAAS polymorphisms modify the phenotype of HCM, particularly MYBPC3-HCM (93,94), and there is now growing evidence that ACE inhibitors, especially combined with low doses of aldosterone receptor blockers, may attenuate the progression of hypertrophy and fibrosis (102–106). In early mouse models of transgenic cardiac troponin T ($cTnT-Q^{92}$) that exhibit myocyte disarray and fibrosis, a randomized, blinded trial comparing losartan (an angiotensin-II blocker) or placebo demonstrated that losartan significantly reversed fibrosis and expression of collagen 1 α (I) and transforming growth factor-beta-1 in the transgenic mice (107). In a similar study involving the same transgenic mice, losartan produced a 50% reduction in myocyte disarray compared with that seen in mice treated with placebo as well as complete normalization of the collagen volume fraction (108).

Lastly, another group of drugs, statins, may favorably modify the phenotype of HCM. A study involving 24 transgenic mice harboring the MYH7-R403Q mutation showed a regression of hypertrophy and fibrosis, improved cardiac function, and reduced *extracellular signal-regulated kinases 1 and 2* activity after treatment with simvastatin compared to 12 nontransgenic mice (109). Similar results were observed in transgenic rabbits with this mutation that were treated with atorvastatin (110). However, a small, randomized controlled pilot study failed to show an effect on humans with HCM (111).

Therefore, one can envision that, with increasing knowledge of the patient's pathogenic substrate and polymorphism profile, specific therapies may someday emerge. In other cases, for example, the proper and prompt recognition of an HCM phenocopy such as cardiac Fabry's disease can facilitate gene-specific pharmacotherapy such as enzyme-replacement therapy. Albeit rare, such clinical sleuthing can

enable early treatment and prevent the progression of the disease. Recently, human trials such as the DELIGHT (Diltiazem Long-term In Genotype-positive Hypertrophic cardiomyopathy as preclinical Treatment) trial have begun and are examining whether calcium-channel inhibitors like diltiazem can prevent the development of hypertrophy among patients with genotype-positive, LVH-negative HCM (112).

Conclusions

Genomic medicine, as it pertains to HCM, has moved from the bench to the bedside, but caution is needed to interpret and manage the genetic portfolio of a patient. Although some prognostic forecasts may be gleaned from the HCM genetic test, therapeutic decisions regarding use of a defibrillator should not be dictated by the genetic test result. Instead, knowledge of the genetic background in subjects with HCM has significant diagnostic implications, and echocardiography may help guide genetic testing by providing anticipatory guidance and a pre-test probability of a positive genetic test result. Clearly, knowledge of disease-causing mutations in an index case enables rapid genetic testing and diagnosis of potentially at-risk relatives thereby providing improved and informed follow-up and treatment decisions for such family members. The information gained in these subjects can define risk status and, in those subjects with negative genetic screening, less close follow-up and testing over time and psychological freedom.

Increasingly, clinical care in HCM and other genetic-based disorders includes the wise use and wiser interpretation of genetic tests. Therefore, understanding the genetic underpinnings of disease and the risk placed on these subjects will be imperative for all patients and their families. The 21st century clinician must be cognizant of the state-of-the-art of translational genetics in order to best care for their patients and families, as well as to help to define new clinical guidelines over the next decade.

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