Genetic basis of hypertrophic cardiomyopathy

Bos, J.M.

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Chapter 1

Genetics of Hypertrophic Cardiomyopathy:
One, Two, or More Diseases?

J. Martijn Bos, Steve R. Ommen, Michael J. Ackerman

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Abstract

Purpose of review. Hypertrophic cardiomyopathy (HCM), affecting 1 in 500 persons, is the most common identifiable cause of sudden death in the young. This review details the history of HCM, recent discoveries in its genetic underpinnings and important genotype-phenotype relationships described in recent studies.

Recent findings. Since the discovery of the genetic underpinnings of hypertrophic cardiomyopathy in 1989 hundreds of mutations scattered amongst at least 10 sarcomeric genes confer the pathogenetic substrate for this “disease of the sarcomere/myofilament”. More recently, the genetic spectrum of HCM has expanded to encompass mutations in Z-disc associated genes (Z-disc hypertrophic cardiomyopathy) and glycogen storage diseases mimicking HCM (metabolic hypertrophic cardiomyopathy). Recent genotype-phenotype studies have discovered an important relationship between morphology of the left ventricle, its underlying genetic substrate and long-term outcome of this disease.

Summary. Genomic medicine has entered the clinical practice and the diagnostic utility of genetic testing for HCM diseases is clearly evident, but with the growing number of hypertrophic cardiomyopathy-associated genes strategic choices have to be made. With recent discoveries in genotype-phenotype relationships, especially pertaining to the echocardiographic septal shape and the underlying pathogenetic mutation, time has come to subdivide the one disease we call HCM.

Keywords

Genetic testing, hypertrophic cardiomyopathy, myofilament, septal morphology
Introduction

Hypertrophic cardiomyopathy (HCM) is a disease of enormous phenotypic and genotypic heterogeneity. Affecting 1 in 500 people, it is the most prevalent genetic cardiovascular disease, and more importantly the most common cause of sudden cardiac death in young athletes[1]. Anatomically/physiologically, HCM can manifest with negligible to extreme hypertrophy, minimal to extensive fibrosis and myocyte disarray, absent to severe left ventricular outflow tract obstruction, and distinct septal contours/morphologies such as reverse curve-, sigmoidal-, and apical variant-HCM. The clinical course varies extremely, ranging from an asymptomatic lifelong course to dyspnea/angina refractory to pharmacotherapy to sudden death as the sentinel event.

HCM was fully described for the first time by Teare in 1958 as 'asymmetrical hypertrophy of the heart in young adults'[2]. It has since been known by a confusing array of names, reflecting its clinical heterogeneity and its uncommon occurrence in daily cardiologic practice. In 1968, the World Health Organization (WHO) defined cardiomyopathies as 'diseases of different and often unknown etiology in which the dominant feature is cardiomegaly and heart failure'[3]. This statement was updated in 1980 and defined cardiomyopathies as 'heart muscles diseases of unknown cause', thereby differentiating it from specific identified heart muscle diseases of known cause, like myocarditis[4].

Throughout the years, names such as idiopathic hypertrophic subaortic stenosis[5], muscular subaortic stenosis[6] and hypertrophic obstructive cardiomyopathy[7] have been widely and interchangeably used to define the same disease. In 1995, a WHO/International Society and Federation of Cardiology Task Force on cardiomyopathies classified the different cardiomyopathies by dominant pathophysiology, or if possible, by etiological/pathogenetic factors[8]. The four most important cardiomyopathies - dilated cardiomyopathy (DCM), restrictive cardiomyopathy (RCM), arrhythmogenic right ventricular cardiomyopathy (ARVC) and HCM - were recognized, next to a number of specific and mostly acquired cardiomyopathies, like ischemic- or inflammatory cardiomyopathy[8].
Accordingly, HCM is described as ‘left and/or right ventricular hypertrophy, usually asymmetric and involving the interventricular septum with predominant autosomal dominant inheritance involving sarcomeric contractile proteins’[8]. This nomenclature has been upheld in the most recent ACC/ESC expert consensus document of 2003[9], although with expanding knowledge on the genetic background of these diseases voices have recently been subclassified into primary cardiomyopathies into genetic -, mixed - and acquired cardiomyopathies[10]. Under this approach, the genetic subgroup entails HCM, ARVC and glycogen storage diseases presenting as HCM, but also includes ion channel disorders such as long QT syndrome (LQTS)[10].

**Genetic background of HCM**

Since the sentinel discovery of the first locus for familial HCM (1989) and the first mutations involving the MYH7-encoded beta myosin heavy chain (1990) as the pathogenic basis for HCM[11, 12], over 300 mutations scattered among at least 24 genes encoding various sarcomeric, calcium handling and mitochondrial proteins have been identified (Table 1). The most common genetically-mediated form of HCM is myofilament-HCM, with hundreds of disease-associated mutations in 8 genes encoding proteins critical to the sarcomere’s thick myofilament [β-myosin heavy chain (MYH7)][12], regulatory myosin light chain (MYL2) and essential myosin light chain (MYL3)][13], intermediate myofilament [myosin binding protein C (MYBPC3)][14], and thin myofilament [cardiac troponin T (TNNT2), α-tropomyosin (TPM1)][15], cardiac troponin I (TNNT3)][16], and actin (ACTC)][17, 18]. Targeted screening of giant sarcomeric TTN-encoded titin, which extends throughout half of the sarcomere, has thus far revealed only one mutation[19]. More recently, mutations have been described in the myofilament protein alpha-myosin heavy chain encoded by MYH6[20]. Although up until 2001 it was thought that specific mutations in these myofilament genes were inherently ‘benign’ or ‘malignant’[21, 22, 23, 24, 25, 26, 27, 28], genotype-phenotype studies involving a large cohort of unrelated patients have indicated that great caution must be exercised with assigning particular prognostic significance to any particular mutation[29, 30, 31].
Furthermore, those studies have demonstrated that the two most common forms of genetically mediated HCM – MYH7-HCM and MYBPC3-HCM – are phenotypically indistinguishable[32]. The prevalence of mutations in the 8 most common myofilament associated genes, currently comprising the commercially available HCM genetic test (www.hpcgg.org) in different international cohorts ranges from 30 to 61%, leaving still a large number of patients with genetically unexplained disease[33].

Over the last few years, the spectrum of HCM-associated genes expanded outside the myofilament to encompass additional subgroups that could be classified as 'Z-disc-HCM', 'calcium-handling HCM', and 'metabolic HCM'; all genes currently implicated in the pathogenesis of HCM are shown in Table 1. As a result of its close proximity to the contractile apparatus of the myofilament and its specific structure-function relationship with regards to cyto-architecture, as well as its role in the stretch-sensor mechanism of the sarcomere, recent attention has been focused on the cardiac Z-disc. Initial mutations were described in muscle LIM protein encoded by CSRP3[34] and telethonin encoded by TCAP[35], an observation replicated in our large cohort of unrelated patients with HCM[36]. LDB3-encoded LIM domain binding 3, ACTN2-encoded alpha actinin 2 and VCL-encoded vinculin/metavinculin have been added to that list[37]. Interestingly, although the first HCM-associated mutation in vinculin was found in the cardiac-specific insert of the gene, yielding the protein called metavinculin[38], the follow up study also identified a mutation in the ubiquitously expressed protein vinculin[39].

As the critical ion in the excitation-contraction coupling of the cardiomyocyte, calcium and proteins involved in calcium induced calcium release (CICR) have always been of high interest in the pathogenesis of HCM. Although with very low frequency, 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 (SERCA)[40, 41] as well as in the RyR2-encoded cardiac ryanodine receptor[42]. Recently, our HCM genetic research program discovered three novel mutations in JPH2-encoded junctophilin 2 in three, previously genotype negative, patients with HCM. This is the first time that JPH2, which is thought to play a role in approximating the sarcoplasmic reticulum calcium release channels and plasmalemmal L-type calcium channels, has been implicated in the pathogenesis of HCM[43].
Table 1: Summary of hypertrophic cardiomyopathy (HCM)-susceptibility genes and the estimated/extrapolated frequency (%) of specific mutations by morphologic subgroup.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Locus</th>
<th>Protein</th>
<th>Reverse Curve HCM</th>
<th>Sigmoidal HCM</th>
<th>Apical HCM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Giant filament HCM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Titin</td>
<td>2q24.3</td>
<td>Titin</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MYH7</td>
<td>14q11.2-q12</td>
<td>β-myosin heavy chain</td>
<td>30 - 40</td>
<td>&lt; 5</td>
<td>10 - 15</td>
</tr>
<tr>
<td>MYH6</td>
<td>14q11.2-q12</td>
<td>α-myosin heavy chain</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MYL2</td>
<td>12q23-q24.3</td>
<td>Ventricular regulatory myosin light chain</td>
<td>&lt; 5</td>
<td>0</td>
<td>2 - 4</td>
</tr>
<tr>
<td>MYL3</td>
<td>3p21.2-p21.3</td>
<td>Ventricular essential myosin light chain</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Intermediate filament HCM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MYBPC3</td>
<td>11p11.2</td>
<td>Cardiac myosin-binding protein C</td>
<td>30 - 40</td>
<td>5</td>
<td>10 - 15</td>
</tr>
<tr>
<td>Thin filament HCM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNNT2</td>
<td>1q32</td>
<td>Cardiac troponin T</td>
<td>5 - 10</td>
<td>&lt;1</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>TNNI3</td>
<td>19p13.4</td>
<td>Cardiac troponin I</td>
<td>1-2</td>
<td>&lt;1</td>
<td>0</td>
</tr>
<tr>
<td>TPM1</td>
<td>15q22.1</td>
<td>α-tropomyosin</td>
<td>1-2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ACTC</td>
<td>15q14</td>
<td>α-cardiac actin</td>
<td>&lt;1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Genes</td>
<td>Chromosomal Location</td>
<td>Function</td>
<td>Z-disc HCM</td>
<td>Calcium handling HCM</td>
<td>Metabolic HCM</td>
</tr>
<tr>
<td>---------------</td>
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<td>---------------</td>
</tr>
<tr>
<td>LBD3</td>
<td>10q22.2-q23.3</td>
<td>LIM binding domain 3 (Alias: ZASP)</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSRP3</td>
<td>11p15.1</td>
<td>Muscle LIM protein</td>
<td>0</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>TCAP</td>
<td>17q12-q21.1</td>
<td>Telethonin</td>
<td>0</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>VCL</td>
<td>10q22.1-q23</td>
<td>Vinculin/metavinculin</td>
<td>0</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>ACTN2</td>
<td>1q42-q43</td>
<td>Alpha-actinin 2</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>RyR2</td>
<td>1q42.1-q43</td>
<td>Cardiac ryanodine receptor</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>JPH2</td>
<td>20q12</td>
<td>Junctophilin-2</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>0</td>
</tr>
<tr>
<td>PLN</td>
<td>6q22.1</td>
<td>Phospholamban</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>PRKAG2</td>
<td>7q35-q36.36</td>
<td>AMP-activated protein kinase</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>LAMP2</td>
<td>Xq24</td>
<td>Lysosome-associated membrane protein 2</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>GLA</td>
<td>Xq22</td>
<td>Alpha-galactosidase A</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>FXN</td>
<td>9q13</td>
<td>Frataxin</td>
<td>-</td>
<td>-</td>
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</tr>
</tbody>
</table>

- Indicates that no genotype-phenotype studies involving a large cohort of unrelated patients have been performed to estimate the frequency of that gene's particular involvement in that particular morphological subtype of HCM.
The last important genetic subgroup of HCM is that of the metabolic HCM, involving mitochondrial and lysosomal proteins. In 2005, Arad et al. first described mutations in lysosome-associated membrane protein-2 encoded by LAMP2 and protein kinase gamma-2 encoded by PRKAG2 in glycogen storage disease-associated genes mimicking the clinical phenotype of HCM[44, 45, 46, 47]. In 2005, a mutation in FXN-encoded frataxin was described in a patient with HCM. Although this patient also harbored a myofilament mutation in MYBPC3-encoded myosin binding protein C, functional characterization showed significant influence of the FXN-mutant on the phenotype, suggesting that the observed alterations in energetics may act in synergy with the present myofilament mutation[48]. Similar to PRKAG2 and LAMP2, Fabry’s disease can express predominant cardiac features of left ventricular hypertrophy. Over the years, mutations in GLA-encoded alpha-galactosidase A have been found in patients with this multi-system disorder [49, 50, 51].

Although up to 24 HCM-susceptibility genes involving different pathways have been identified, the search for novel mutations in new genes continues. Recently, a genome wide-linkage study identified a new locus for HCM in a large family with left ventricular hypertrophy located to chromosome 7. Subsequent studies of genes located to this region however have thus far not yielded the causative gene[52]. As a result of the increasing genetic heterogeneity of HCM, a classification based on functional genetics might seem very helpful, but in light of the low yield of mutations in a large number of these genes as well as the commercial availability of just a small number of these genes, a phenotypic classification might be a more useful tool in looking at this disease from a clinical practice vantage point.
Genotype-phenotype analyses in HCM

Numerous studies have tried to identify phenotypic characteristics most indicative of myofilament/sarcomeric-HCM to facilitate genetic counseling and strategically direct clinical genetic testing[29, 31, 32, 53, 54, 55, 56]. Although several phenotype-genotype relationships have emerged to enrich the yield of genetic testing, these patient profiles have not been particularly clinically informative. An important discovery, linking the echocardiographically determined septal morphology to the underlying genetic substrate, was recently made.

The first link to be drawn between septal morphologies was a result of HCM study by Lever and colleagues in the 1980s, in which septal contour – classified as reverse septal contour, sigmoidal septal contour, apical - and neutral contour - was found to be age-dependent with a predominance of sigmoidal-HCM being present in the elderly[57]. In the early 90’s Seidman et al described an early genotype-phenotype observation involving a small number of patients and family members and discovered that patients with mutations in the beta myosin heavy chain (MYH7-HCM) generally had reversed curvature septal contours (reverse curve-HCM)[58].

Inspired by these two initial observations, we recently finished a large genotype-phenotype analysis correlating the septal morphology with the underlying genotype. After extensive analysis of the echocardiograms of 382 previously genotyped and published patients[32, 53, 56], we observed that sigmoidal-HCM (47% of cohort) and reverse curve-HCM (35% of cohort) were the two most prevalent anatomical subtypes of HCM, and discovered that the septal contour was the strongest predictor for the presence of a myofilament mutation, regardless of age [59]. Multivariate analysis in this cohort demonstrated septal morphology was the only independent predictor of myofilament HCM with an odds ratio of 21 (p<0.001), when reverse curve morphology was present[59]. Apical HCM, in which the hypertrophy is mostly concentrated around the apex of the heart, was found in 10% (n=37) of our cohort. The yield of the commercially available HCM genetic test for myofilament-HCM was 79% in reverse curve-HCM but only 8% in patients with sigmoidal-HCM. Of the smaller subgroup of patients with apical HCM, 32% had a mutation in one of the myofilaments [56].
These observations may facilitate echo-guided genetic testing by enabling informed genetic counseling about the a priori probability of a positive genetic test based upon the patient’s expressed anatomical phenotype (Figure 1). In addition, the paucity of myofilament mutations in sigmoidal-HCM opens the door for research to elucidate the molecular/genetic determinants of sigmoidal HCM.

Figure 1: Functional subgroups of genetic hypertrophic cardiomyopathy (HCM) and the yield of genetic testing for the two most common septal morphologies with their respective subgroup. Shown are the most important functional subgroups of genetically mediated HCM and the yield of mutations over various cohorts. Blue arrows indicate the functional relationship between the different elements. The black arrows show the yield of genetic testing for the subgroups of myofilament HCM and Z-disc HCM and their morphologic subgroups. LAMP2, lysosome-associated membrane protein 2; PLN, phospholamban; PRKAG2, AMP-activated protein kinase; SR, sarcoplasmic reticulum; RyR2, cardiac ryanodine receptor.
With the majority of known myofilament proteins studied, except for a complete analysis of the giant protein \textit{TNN}-encoded titin, recent research has been focused on proteins beyond the cardiac myofilaments, especially proteins involved in the cytoarchitecture and cardiac stretch sensor mechanism of the cardiomyocyte localized to the cardiac Z-disc (Figure 1). The Z-disc is an intricate assembly of proteins at the Z-line of the cardiomyocyte sarcomere. Extensively reviewed, proteins of the Z-disc are important in the structural and mechanical stability of the sarcomere as they appear to serve as a docking station for transcription factors, calcium signaling proteins, kinases and phosphatases [60, 61]. In addition, this assembly of proteins seems to serve as a way station for proteins that regulate transcription by aiding in their controlled translocation between the nucleus and the Z-disc[60, 61].

With all of these roles, a main implication for the Z-disc is its involvement in the cardiomyocyte stretch sensing and response systems[62]. Mutations in three such proteins localized to the cardiac Z-disc, \textit{CSRP3}-encoded muscle LIM protein (MLP), \textit{TCAP}-encoded telethonin and \textit{VCL}-encoded vinculin, including its cardiac specific insert of exon 19 that yields metavinculin, have previously been established as both HCM[34, 35, 36, 38, 39] and dilated cardiomyopathy (DCM)-susceptibility genes[34, 35, 36, 38, 63, 64]. Additionally, it is now fully appreciated that these divergent cardiomyopathic phenotypes of HCM and DCM are partially allelic disorders with \textit{ACTC}, \textit{MYH7}, \textit{TNNT2}, \textit{TPM1}, \textit{MYBPC3}, \textit{TNT}, \textit{MLP}, \textit{TCAP}, and \textit{VCL} established as both HCM- and DCM-susceptibility genes[34, 35, 38, 63, 65, 66, 67, 68, 69].

Mutations in \textit{ACTN2}-encoded alpha-actinin-2 (ACTN2) and \textit{LDB3}-encoded LIM domain binding 3 (LDB3) as novel HCM-susceptibility genes[37] were described. Building on our discovery linking reverse-curve HCM to the presence of myofilament mutation, and recognizing that the Z-disc may transduce multiple signaling pathways during stress, translating into hypertrophic responses, cell growth and remodeling [70], we have observed that Z-disc HCM, in contrast to myofilament HCM, is preferentially sigmoidal. Eleven out of 13 patients with Z-disc HCM had a sigmoidal septal contour and no reverse septal curvatures were seen [37]. We speculate that Z-disc HCM leads to a hypertrophic response that is expressed in the areas of highest stress (i.e. LVOT) and therefore predisposes to a sigmoidal septal contour.
Intriguing conclusions can be drawn from these observations. Whereas in initial morphologic studies, sigmoidal-HCM seemed to be associated with older age [57], the underlying genotype rather than age appears to be the predominant determinant of septal morphology [59]. Furthermore, Z-disc HCM seems to have a predilection for sigmoidal contour status. Given that the vast majority of our patients with sigmoidal HCM still lack a putative disease-causing mutation, the molecular underpinnings responsible for a sigmoidal morphology remain to be elucidated. Alternatively, it seems plausible that a HCM-predisposing mutation might not be the principle determinant for many patients with sigmoidal-HCM. Instead, a multi-factorial model may be responsible for this subtype of clinically diagnosed HCM.

In this model, the sum of all contributors – the presence or absence of a mutation or LVH promoting polymorphisms [71], an unidentified genetic substrate, environmental factors and hypertension, culminates in what is clinically labeled as HCM. This multi-factorial model for sigmoidal-HCM is supported by the significantly older age at diagnosis of patients with sigmoidal-HCM (49 years) compared to those with reverse curve-HCM (32 years) [59] and the fact that nearly 20% of patients classified with sigmoidal-HCM were noted to have mild hypertension [59]. Diagnosed with HCM by experienced physicians, a subset of this group may have a basal septum more sensitive to the pro-hypertrophy trigger of increased afterload, precipitating basal septal hypertrophy (sigmoidal disease).
Conclusions

Genomic medicine has entered the clinical practice as it pertains to the evaluation and management of HCM. The diagnostic utility of genetic testing for HCM diseases is clearly evident, but strategic choices have to be made with the growing number of genes implicated in this disease. With recent discoveries in genotype-phenotype relationships, especially pertaining the echocardiographic septal shape and the underlying pathogenetic mutation, time has come to further subdivide the one disease we call HCM.

Clinical HCM specialists are accustomed already to prefacing the HCM label with physiological descriptors of obstructive- and non-obstructive-HCM and anatomical/morphological descriptors: reverse curve-, sigmoidal-, and apical-HCM. Accordingly, a pathogenetic subdivision seems warranted. Just as there is no prerequisite for clinically diagnosed HCM to necessarily be obstructive or reverse curve in nature, it should not be mandated that clinically diagnosed HCM requires a genetic perturbation in one of the sarcomeric myofilaments. Instead, what is emerging is a clear picture that the two most common anatomical/morphological subtypes of HCM (reverse curve- and sigmoidal-HCM) largely emanate from fundamentally distinct pathogenetic mechanisms. Herein, most (but not all) of reverse curve-HCM is indeed a "disease of the sarcomere" and most (but not all) sigmoidal-HCM is in search of its etiology.
References


