

# Clinical and Molecular Genetic Aspects of Hypertrophic Cardiomyopathy

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**Abstract:** Hypertrophic cardiomyopathy (HCM) is a fascinating disease with diverse phenotypic expression that spans from minimal hypertrophy to severe heart failure and sudden cardiac death (SCD). HCM is the most common cause of sudden cardiac death (SCD) in the young competitive athletes and a major cause of morbidity and mortality in elderly. Molecular genetic basis of HCM is all but elucidated by identification of several hundred different mutations in 11 different genes, all encoding sarcomeric proteins. The emphasis of current research is to develop genetic screening techniques in order to identify the mutation carriers prior to and independent of the clinical manifestations of HCM; to identify genetic and non-genetic determinants of clinical outcome in HCM; and to identify novel drug targets in order to prevent, attenuate or reverse the evolving phenotype. Recent studies have led to partial understanding of the molecular pathogenesis of HCM phenotypes and consequently, identification of new therapeutic targets, which have been tested in animal models of human HCM with promising results. Studies in transgenic animal models have shown the treatment with statins or blockade of renin-angiotensin-aldosterone system could attenuate and mitigate evolving cardiac phenotypes in HCM. Studies in human patients with HCM are needed to determine whether the observed beneficial effects of new pharmacological interventions also extend to humans with HCM.

**Key words:** Hypertrophic cardiomyopathy, sudden death, genetics, mutations, sarcomere, phenocopy, treatment, heart failure.

## INTRODUCTION

Hypertrophic cardiomyopathy (HCM), a primary disease of the myocardium, has fascinated many investigators throughout the last century. HCM was first noted by two French pathologists in the 19th century and its gross pathology, with an emphasis on narrowing of the left ventricular outflow tract, was described by a German pathologist in 1907. The disease remained largely unrecognized as a clinical phenotype until the second half of the 20<sup>th</sup> century, when the initial reports in 1950s detailed pathological phenotypes along with the familial inheritance. Subsequent description of HCM phenotypes paralleled the development of modern diagnostic tools. In 1960s, advances in cardiac catheterization led to the diagnosis of outflow tract obstruction and hence, HCM was described primarily as a hemodynamic entity characterized by subaortic stenosis. The term "idiopathic hypertrophic subaortic stenosis or IHSS" was commonly used to describe HCM during this period. The first echocardiographic report appeared in late 60s and followed by extensive reporting on the asymmetric nature of hypertrophy during 1970s. The term asymmetric septal hypertrophy (ASH) was coined to emphasize the predominant involvement of the inter-ventricular septum in HCM. The routine application of Doppler echocardiography during 1980s replaced cardiac catheterization for the detection and quantification of left ventricular outflow tract obstruction and led to a better appreciation of diastolic dysfunction. The era of molecular genetics arrived in 1990s upon discovery of the first causal gene and mutation for HCM [1]. Since then, a large number of genes and mutations

have been identified and the genetic basis of HCM is all but delineated. The current emphasis is to decipher the molecular pathogenesis of HCM and to develop pharmacological intervention in order to prevent, attenuate or reverse the evolving phenotype. One would anticipate that within the next few years, advances in the molecular genetics and biology of HCM lead to an early diagnosis of mutation carriers and risk stratification prior to and independent of the clinical phenotypes and ultimately, development of new therapies based on blockade or activation of specific pathways involved in the pathogenesis of clinical manifestations of HCM. The ultimate goal of correcting the genetic defect had to wait the development of specific strategies, whereby a single base pair could be changed with a high fidelity.

## DEFINITION

HCM is a primary disease of the myocardium characterized by left ventricular hypertrophy in the absence of an increased external load - "unexplained cardiac hypertrophy" -, a hyperdynamic left ventricle and a small left ventricular chamber. The diagnosis of HCM, based on clinical criteria, however, is not sufficiently specific as "unexplained cardiac hypertrophy" could also occur in storage diseases, mitochondrial diseases, Fabry disease, triplet repeat syndromes, sarcoidosis, and many others [2]. Whether the definition of HCM should be restricted to specify the phenotype arising from defective sarcomeric proteins and be exclusive of other forms of "unexplained cardiac hypertrophy" remains to be settled. The latter forms of cardiac hypertrophy are often referred to as HCM phenocopy, which implies a phenotype grossly similar to HCM but not truly HCM. Clinically, patients with HCM phenocopy are often diagnosed as true HCM patients, as the gross phenotypes are often indistinguishable [3]. Certain

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features could help to distinguish patients with true HCM, such as hyperdynamic left ventricle and small left ventricular chamber size. In contrast, in HCM phenocopy, global cardiac systolic function is often decreased. Perhaps, the most remarkable diagnostic feature is the presence of myocyte disarray, which is considered the pathological hallmark of HCM and hence, is expected to be absent in HCM phenocopy. Other notable features of HCM phenocopy is the presence of other cardiac and non-cardiac concomitant phenotypes, such as conduction defects, deafness, neurological abnormalities, and skeletal myopathy. Finally, HCM phenocopy often arises from storage disorders, such as Fabry disease, Pompe disease, which have their additional phenotypes. Mechanistic studies also illustrate the differences in the pathogenesis of HCM and HCM phenocopy, as shown for HCM phenocopy arising from mutations in the  $\beta$  subunit of adenosine monophosphate kinase, which is characterized by cardiac hypertrophy because of deposition of glycogen in the heart, conduction defect, and pre-excitation pattern on electrocardiogram [4-6].

### PREVALENCE

The prevalence of HCM, defined as echocardiographic wall thickness of 15 mm or greater in the absence of a secondary cause, in 23 to 35 -year old individuals is approximately 1:500 [7]. A similar prevalence has been estimated in hospital survey of Japanese patients [8]. These estimates are relatively conservative because they are restricted to individuals expressing cardiac hypertrophy and even so, many patients with HCM have milder degree of left ventricular hypertrophy and because of age-dependent penetrance of the causal mutations. The estimates are not necessarily specific because of possible phenocopy. Age-dependence of penetrance would indicate a higher prevalence in the older population. Another confounding issue in estimating the prevalence of HCM in the elderly is the high prevalence hypertension, which, by definition, leads to exclusion of such as patients as having HCM. However, it is noteworthy that hypertension and HCM could exist concomitantly and the entity referred to as "hypertensive hypertrophic cardiomyopathy of elderly" may indeed reflect concomitant presence of hypertension and HCM [9].

### PHENOTYPIC EXPRESSION OF HCM

HCM is a great masquerader with a diverse array of clinical, morphological, and pathological phenotypes. Indeed, a major feature of HCM is variability of its clinical manifestations, which in conjunction with the relatively low prevalence of HCM, often leads to conflicting results in different studies of HCM [10, 11]. The majority of patients with HCM are asymptomatic or minimally symptomatic. The most common symptoms are those of (diastolic) heart failure, chest pain, palpitations, dizziness, and lightheadedness. Syncope is an infrequent but a serious symptom, as it is often associated with cardiac arrhythmias and heralds sudden cardiac death (SCD) [12-14]. Atrial fibrillation and non-sustained ventricular tachycardia are the predominant cardiac arrhythmias in HCM and are associated with adverse clinical outcome [15, 16]. Electrocardiographic findings of Wolff-Parkinson-White syndrome are present in a relatively small percentage of patients with clinically apparent HCM, which the recent data suggests may be a

phenocopy and not a true HCM [4]. SCD is often the first and tragic manifestation of HCM in apparently young healthy individuals [17, 18]. HCM is considered the most common cause of SCD in the young competitive athletes [17]. The risk of SCD is greater during or immediately after exercise. Several clinical, pathological and genetic factors have been associated with the risk of SCD in HCM, however, none alone has a strong positive predictive value and the global risk, derived from the combination of all known risk factors, should be considered in counseling and managing patients with HCM [14, 19]. Table 1 lists potential risk factors for SCD in patients with HCM. Overall, HCM is considered a relatively benign disease with an annual mortality of about 1% in the adult population [20, 21].

**Table 1.**

| Potential Risk Factors for SCD in Patients with HCM            |
|--|
| Causal mutations and modifier genes                            |
| Previous history of aborted SCD                                |
| Family history of SCD (more than 1 SCD victim in the family)   |
| History of syncope   |
| Severe hypertrophy   |
| Significant outflow tract gradient                             |
| Severe interstitial fibrosis and myocyte disarray              |
| Sustained and repetitive non-sustained ventricular tachycardia |
| Early onset of clinical manifestations (young age)             |
| Abnormal blood pressure response to exercise                   |
| Presence of myocardial ischemia                                |

Cardiac hypertrophy is the quintessential feature of HCM and the basis for its clinical diagnosis. Hypertrophic is concentric, leading to a small left ventricular chamber size. It is asymmetric in 2/3 of the cases with septum being the predominant site of involvement. Hypertrophy may be localized to apex of the heart, which leads to a variant known as apical HCM; or the lateral wall and rarely, the posterior wall only. Morphologically cardiac hypertrophy in HCM has been classified into 4 types, according to the site and extent of involvement of the left ventricular walls [22]. Apical HCM is an uncommon form characterized by giant T wave inversion in the precordial leads of the electrocardiogram and carries a relatively benign prognosis with a 15-year survival rate of approximately 95% in the North American population [23]. As regards cardiac function, left ventricular ejection fraction, a global index of systolic function, is increased or at least preserved. In a small fraction of patients with HCM, the phenotype evolves into dilated cardiomyopathy (DCM) with systolic heart failure. The main functional abnormality in HCM is diastolic dysfunction, which is the primary reason for symptoms of heart failure and morbidity.

The basis for an increased left ventricular ejection fraction in HCM is unclear, but generally has been

interpreted to indicate enhanced myocardial contractility. However, ejection fraction is a load-dependent index and because of a smaller left ventricular end-diastolic volume due to concentric hypertrophy, afterload is reduced in HCM [24, 25]. Assessment of myocardial function using tissue Doppler or magnetic resonance imaging studies also suggest reduced myocardial systolic and diastolic function [26, 27]. Notably, reduced tissue Doppler velocities of the myocardium precede the development of cardiac hypertrophy and could serve as a possible early diagnostic marker for HCM [28]. However, it remains to be determined whether reduced tissue Doppler velocities or magnetic resonance parameters of cardiac function, are reflective of myocardial contractility or are secondary to structural abnormalities of the heart. Another reason for an increased ejection fraction is enhanced  $Ca^{+2}$  sensitivity of myofibrils, observed for a few HCM mutation, which would explain the basis for enhanced contractility in some but not all HCM cases [29]. Finally, prolongation of left ventricular ejection time, which will lead to a smaller left ventricular end systolic volume and hence, a greater ejection fraction, is another possible explanation for an increased left ventricular ejection fraction, as observed in a transgenic mouse model of human HCM mutation [30]. The molecular basis for the prolongation of left ventricular ejection time is unclear. It probably reflects the inability of the thick and thin filaments comprising the mutant sarcomeric protein to dissociate following ATPase hydrolysis during a cardiac cycle. The latter could occur because of lower ADP concentration due to impaired ATPase activity or simply because of altered moiety of sarcomeric proteins interactions.

Pathological features of HCM encompass cardiac myocyte hypertrophy and disarray, interstitial fibrosis and thickening of media of intra-mural coronary arteries among the others. Myocyte disarray is considered the pathological hallmark of HCM and often comprises more than 20% of the ventricle, as opposed to < 5% of the myocardium in the normal hearts [31, 32]. Myocyte disarray is also more prominent in the inter-ventricular septum, but commonly present throughout the myocardium [32]. Cardiac hypertrophy, interstitial fibrosis, and myocyte disarray are associated with the risk of SCD, mortality and morbidity in patients with HCM [33-36].

## MOLECULAR GENETIC BASIS OF HCM

HCM is a genetic disease with an autosomal dominant mode of inheritance. Familial HCM comprises approximately half of the cases. In the remainder, HCM is sporadic. Despite description of familial HCM more than 50 years ago, elucidation of its molecular genetic basis had to await the development of modern genetic techniques. In 1999, Dr. Seidman's group identified the first causal mutation for HCM, namely; the R403Q mutation in the  $\beta$ -myosin heavy chain (MyHC) [1]. This seminal discovery led to subsequent identification of over 300 causal mutations in 11 different genes, all encoding sarcomeric proteins (Table 2) [37]. Accordingly, HCM is considered a disease of mutant contractile sarcomeric proteins [38]. However, as discussed above, the phenotype of cardiac hypertrophy in the absence of an increased external load is not unique to HCM but also occurs in storage diseases, mitochondrial disorders and

triplet repeat syndromes (Table 2). As discussed earlier, such entities, while sometimes grossly identical to sarcomeric HCM, are considered phenocopy but not true HCM.

Based on the available data from genetic epidemiological studies, it is estimated that approximately 80% of the causal genes and mutations have already been identified [11, 37]. The most common causal genes are the *MYH7*, *MYBPC3*, *TNNT2* and *TNNI*, which encode the  $\beta$ -MyHC, myosin binding protein-C, cardiac troponin T and cardiac troponin I, respectively. Collectively, mutations in the above genes account for approximately 2/3 of all HCM cases [37, 39]. *MYH7* and *MYBPC3* are responsible each for approximately a third of all HCM cases, while *TNNT2* and *TNNI* are less common, each being responsible for approximately 10-15% of all cases. Over 100 different mutations in the  $\beta$ -MyHC have been identified, the vast majority of which are missense mutations located in the globular head of  $\beta$ -MyHC [40]. Mutations in the hinge and rod domain of  $\beta$ -MyHC also have been described but in general, are less common [37, 39]. Similarly, over 100 different mutations in the *MYBPC3* have been identified that are scattered through the genes [9, 41]. A large number of mutations in MyBP-C, unlike the  $\beta$ -MyHC mutations, are insertion/deletion or splice junction mutations [9, 41]. Such mutations by changing the reading frame of the encoded protein could lead to expression of a truncated MyBP-C protein, reduce expression level of the MyBP-C protein, or severely impair function of the encoded protein [41]. The frequency of each particular mutation in the *MYH7* and *MYBPC3* is relatively low and no specific mutation predominates. Nonetheless, a few hot spots for mutations, such as codons 403 and 719 in *MYH7* have been identified [42, 43].

*TNNT2* and *TNNI3* genes encoding cardiac troponin T and I, respectively, are relatively uncommon causes of HCM. Over 20 mutations in *TNNT2* and over 20 mutations in *TNNI3* have been identified, the vast majority of which are missense mutations. Mutation in cardiac troponin T and I, components of the thin filaments of the sarcomeres, collectively are responsible for approximately 10 to 15% of all HCM cases [37, 39]. As is the case for other causal genes for HCM, the frequency of each specific mutation is relatively low and the presence of common ancestral allele in HCM is rare. Deletion mutations in troponin T that involve the splice donor sites and could lead to truncation of the encoded proteins also have been reported [38].

Other known causal genes for HCM are genes encoding  $\beta$ -tropomyosin (*TPM1*), titin (*TTN*), cardiac  $\alpha$ -actin (*ACTC*), essential and regulatory light chains (*MYL3* and *MYL2*, respectively), myosin light chain kinase 2 (*MYLK2*), and perhaps, cardiac troponin C (*TNNC1*) and  $\beta$ -MyHC [39]. Mutations in these genes are uncommon and collectively account for approximately 10% of all known mutations.

## Modifier Genes

Clinical manifestations of HCM are quite variable not only among individuals with different causal mutations but also among members of a single family or members of different families with identical causal mutations [44]. The basis for the inter-individual variability in the phenotypic expression of HCM is largely unknown. Nevertheless, it is

Table 2.

| <b>List of Causal Genes for HCM</b>   |                   |                    |  |                  |
|---|-------------------|--------------------|--|------------------|
| <b>Locus</b>  | <b>Chromosome</b> | <b>Gene Symbol</b> | <b>Gene</b>  | <b>Frequency</b> |
| <b>Contractile sarcomeric Proteins (true HCM)</b>   |                   |                    |  |                  |
| CMH1  | 14q12             | <i>MYH7</i>        | -Myosin heavy chain                                | ~30%             |
| CMH2  | 1q32              | <i>TNNT2</i>       | Cardiac troponin T                                 | ~ 5 - 10%        |
| CMH3  | 15q22.1           | <i>TPM1</i>        | -tropomyosin                                       | < 5%             |
| CMH4  | 11p11.2           | <i>MYBPC3</i>      | Myosin binding protein-C                           | ~ 30%            |
| CMH5  | 11q               | <i>ACTA</i>        | Cardiac -actin                                     | <5%              |
| CMH7  | 19p13.2           | <i>TNNI</i>        | Cardiac troponin I                                 | ~5 – 10%         |
| CMH8  | 3p21.3-p21.2      | <i>MYL3</i>        | Essential myosin light chain                       | <5%              |
| CMH9  | 2q24.3            | <i>TTN</i>         | Titin  | <5%              |
| CMH10   | 12q23.q24.3       | <i>MYL2</i>        | Regulatory myosin light chain                      | <5%              |
|   | 14q12             | <i>MYH6</i>        | -Myosin heavy chain                                | Rare             |
|   | 20q13.3           | <i>MYLK2</i>       | Cardiac myosin light peptide kinase                | Rare             |
|   | 3p21.3-3p14.3     | TNNCI              | Cardiac troponin C                                 | Probable         |
| <b>Non-sarcomeric proteins Causing HCM (? Phenocopy, also associated with other phenotypes)</b> |                   |                    |  |                  |
| CMH6  | 7q22-q31.1        | <i>PRKAG2</i>      | Protein Kinase A, subunit                          | Rare             |
|   | 6q12              | <i>MOY6</i>        | Unconventional myosin 6                            | Rare             |
|   | MtDNA             | <i>MTTG, MTTI</i>  | Mitochondrial Genes                                | Rare             |
|   | 9q13              | <i>FRDA</i>        | Frataxin (Friedreich' ataxia)                      | Rare             |
|   | 19q13             | <i>DMPK, DMWD</i>  | Myotonic Protein kinase (Myotonic dystrophy)       | Uncommon         |
|   | 12q24             | <i>PTPN11</i>      | Protein tyrosine phosphatase, non receptor type 11 | Rare             |

evident that the causal mutations alone do not fully account for the remarkable variability that exists among the affected members with HCM. Recent data suggests that the presence of multiple mutations could account for part of the variability, however, multiple mutations, being present in less than 10% of the affected members, are uncommon and alone insufficient to explain fully the variability [45]. Thus, factors other than the causal genes and mutations contribute to expression of cardiac phenotypes in HCM.

It is now generally accepted that single nucleotide polymorphisms (SNPs) in the human genome form the backbone of the inter-individual variability in susceptibility to disease, response to therapy and the clinical outcome. In addition, SNPs and other polymorphisms in the genome affect phenotypic expression of single gene disorders, such as HCM. Accordingly, the phenotypic expression of HCM is determined not only by the causal mutations but also by the genetic backgrounds of the affected individuals. SNPs in genes implicated in cardiac growth and hypertrophy are expected to affect the phenotypic expression of cardiac hypertrophy and account for the inter-individual variation in the severity of the disease. Genes with functional variants that affect phenotypic expression of single gene disorders are

referred to as the modifier genes [46]. Modifier genes are not to be confused with the causal genes or mutations, as they are neither necessary nor sufficient to cause HCM, while the causal mutations are necessary and sufficient to cause HCM. Thus, the modifier genes, in the presence of causal genes, only affect the severity of phenotypes, such as the magnitude of cardiac hypertrophy and the risk of SCD. In general, a large number of modifier genes are expected to affect the phenotypic expression of HCM, each imparting a relatively small effect. The first potential modifier gene for human HCM was the angiotensin-1 converting enzyme 1 (ACE-1) gene, as it was shown that its variants were associated with the severity of cardiac hypertrophy and the risk of SCD [47-49]. Several other candidates also have been identified [50]; however, the results have been largely provisional pending replication and confirmation through experimentation. Overall, the identity of the vast majority of the modifier genes in HCM and the magnitude of their effects remain largely unknown and subject to ongoing investigations.

#### **GENOTYPE-PHENOTYPE CORRELATION**

Genetic factors, whether causal or modifier, are important determinants of phenotypic expression of cardiac hypertrophy and the risk of SCD in patients with HCM [41,

47, 48, 51-57]. Causal mutations are considered the primary determinants and the modifier genes as the secondary determinants of phenotypes. Nonetheless, as discussed earlier, there is a remarkable inter-individual variability and accordingly, it has been difficult to generalize the findings or extend the results observed in one group to another. As is the case for all indices of prognosis, whether genetic or clinical, caution is necessary when applying the results of the group data to a single individual. Despite these limitations, however, the existing data suggests that mutations in the  $\beta$ -MyHC are associated with an early onset and more extensive hypertrophy and a relatively higher incidence of SCD [9, 58]. This is in contrast to mutations in the MyBP-C, which, in general, are associated with a late onset and a relatively mild degree of cardiac hypertrophy [9, 41, 58]. Accordingly, the penetrance of mutations in the *MYBPC3* gene is lower than that of mutations in the *MYH7*. The relatively low penetrance of certain mutations in HCM indicates that normal physical examination, electrocardiogram and echocardiogram do not exclude possible presence of the causal mutation, particularly in the young, since penetrance is age-dependent and increases with aging. The clinical implication of a relatively low penetrance is that those at risk should be evaluated periodically, as some individual will develop the phenotype later in life [59]. The phenotypes induced by mutations in cTnT and cTnI are also variable but in general, these mutations are associated with a mild degree of cardiac hypertrophy, but a higher incidence of SCD and extensive myocyte disarray [36, 55]. Overall, the topography of the causal mutations, and hence, their impact on the structure and function of the respective proteins, is expected to be an important determinant of the phenotype. This is perhaps, best illustrated in the case of mutations in cardiac troponin T, troponin I and the  $\beta$ -MyHC genes, wherein mutations, probably based on their topographic location, can cause either HCM or DCM [60, 61]. Mutations enhancing  $Ca^{+2}$  sensitivity of myofibrillar force generation or reducing the inhibitory effects of cTnI on acto-myosin interactions are expected to cause HCM. In contrast, mutations reducing  $Ca^{+2}$  sensitivity of myofibrillar force generation and strengthening the inhibitory effect of cTnI on acto-myosin interaction are expected to cause DCM [62-64]. However, no systematic genotype-phenotype correlation study, based on topographic classification of the mutations, has been performed. Finally, it is important to note that there is significant variability in the phenotypic expression of mutations and “benign” or “malignant” mutations in HCM causal genes have been described.

Another factor that determines the phenotypic expression of HCM is the presence of concomitant diseases, such as hypertension that by providing additional stimulus for hypertrophy could accelerate the phenotypic expression of HCM and the penetrance. This is best exemplified in the case of “hypertensive hypertrophic cardiomyopathy of the elderly”, which is considered a form of HCM caused by mutations in the MyBP-C and its phenotypic expression enhanced because of concomitant hypertension [9]. The tragic SCD of young competitive athletes has raised the intriguing question of whether physical exercise affects phenotypic expression of HCM, such as the risk of SCD and the severity of cardiac hypertrophy. The issue is largely

unsettled. However, based on our understanding of the pathogenesis of HCM indicating that hypertrophy is a secondary phenotype, it is expected that any factor that increases cardiac load and provides a stimulus for cardiac growth, to enhance phenotypic expression of HCM. The impact is expected to be greater for iso-metric exercises, such as weight lifting than isotonic exercises. In view of the increased risk of SCD during or immediately after exercise and given the potential contribution of heavy exercise to the phenotypic expression of HCM, it is generally recommended that individuals at risk to avoid heavy physical activities and competitive or contact sports.

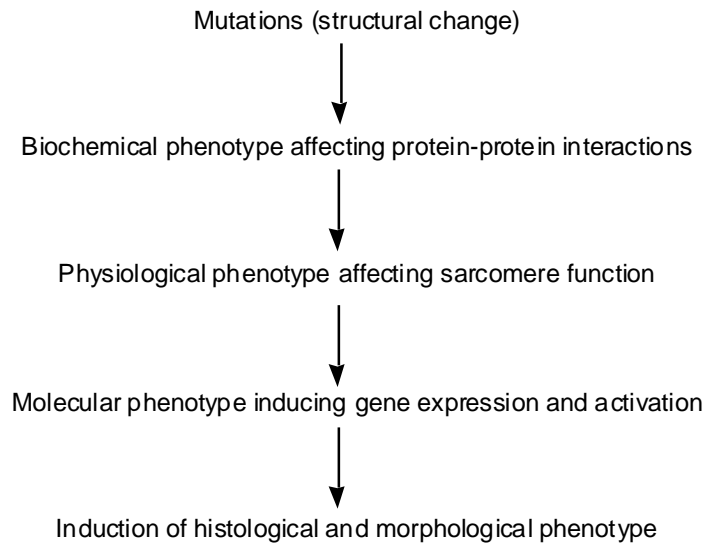
## PATHOGENESIS OF HCM

Delineation of the molecular pathogenesis of HCM has led a series of *in vitro* and *in vivo* experiments to elucidate the molecular pathogenesis of HCM. The results have shown the diversity of the initial defects induced by the causal mutations and the involvement of multiple independent and inter-dependent pathways in the pathogenesis of clinical and pathological features of HCM. Collectively, these studies have led to partial understanding in the links between the causal genetic defect and the phenotypic expression of hypertrophy, fibrosis, and myocyte disarray in HCM.

The diversity of the initial defects is partially reflective of the diversity of the causal genes and mutations. In the pathogenesis of HCM, the initial phenotypes are considered largely structural at the molecular levels, affecting interactions between amino acids or domains of the mutant protein or the mutant protein with other protein components of the sarcomeres and the related proteins. Impaired molecular interactions could lead to functional phenotypes, such as reduced ATPase activity and impaired association and dissociation of the thick and thin filaments and generation of force of contraction or relaxation during a cardiac cycle [65]. Subsequently or in conjunction with the biochemical phenotypes, intermediary molecular phenotypes, such as activation of intracellular signaling molecules, are expressed, which collectively instigate the induction of structural and histological phenotypes, such as hypertrophy, fibrosis, and disarray, among others (Fig. 1). Accordingly, cardiac hypertrophy, the clinical hallmark of HCM, is considered a secondary or compensatory phenotype and hence, potentially reversible [65]. The primary functional stimulus for the subsequent development of cardiac hypertrophy is not known but impaired myocardial contraction and bioenergetics have been implicated [66-69]. In support of myocardial dysfunction as the stimulus for induction of hypertrophy and fibrosis, we have shown that tissue Doppler velocities of myocardial contraction and relaxation were reduced in human subjects with the HCM mutations prior to the development of cardiac hypertrophy [26]. Similarly, impaired myocardial bioenergetics has been implicated as an initial defect, as evidenced by the reduced ratio of cardiac phosphocreatine (PCr) to adenosine triphosphate (ATP) in the heart [70]. Whether these initial phenotypes are unique to specific mutations or common to all, remains to be settled.

It is also notable that the primary phenotype imparted by mutations in troponin T and I, which are primarily responsible for the  $Ca^{+2}$  mediated acto-myosin interactions

### **Sequence of Events in the Pathogenesis of HCM**



**Fig. (1).**

during cardiac cycle, is likely to involve the  $\text{Ca}^{+2}$  responsiveness of the myofibrils [29, 62, 71]. The emerging clinical phenotype could depend on the effects of the causal mutations on  $\text{Ca}^{+2}$  sensitivity of the myofibrillar force generation and ATPase activity. Accordingly, mutations leading to enhance sensitivity cause HCM and in contrast, those leading to reduced sensitivity cause DCM [63]. Whether and how the enhanced  $\text{Ca}^{+2}$  sensitivity of myofibrillar ATPase activity or force generation alone could lead to cardiac hypertrophy, interstitial fibrosis or myocyte disarray remains unclear.

A number of the causal mutations in HCM are splice-junction or frame-shift mutations that could lead to a null-allele effect. In these situations the mutant sarcomeric proteins are not expressed or if expressed are not incorporated into myofibrils. In either case, the net result is “haplo-insufficiency”, which is particularly relevant to frame-shift or insertion-deletion mutations in the MyBP-C protein [41, 72, 73]. Whether “haplo-insufficiency” could change the stoichiometry of the sarcomeric proteins and whether compensatory mechanisms are sufficient to compensate for the null-allele are unclear. The results of gene-targeting experiments in mice provide some credence for the haplo-insufficiency hypothesis, as ablation of one copy of the  $\beta$ -MyHC gene leads to alteration in sarcomeric structure and myocardial dysfunction [74]. However, “haplo-insufficiency” may be gene specific, as ablation of  $\beta$ -tropomyosin did not lead to discernible morphological or functional abnormalities in mouse [75, 76]. Thus, the null mutations may lead to HCM, only when compensatory mechanisms fail to overcome the haplo-insufficiency.

Whether the causal mutations are gain or loss of function mutations is somewhat controversial and probably varies for the different mutations. First, the definition of gain or loss of function is somewhat arbitrary, as the mutation effect could

differ at the structural, biochemical, and biophysical levels. In general, any deviation from a normal state could be considered as a “loss of function”. Accordingly, one could consider an enhanced affinity of the mutant  $\beta$ -MyHC protein for the actin filaments as a gain of function, while as the same token, such enhanced affinity could slow the dissociation of the mutant  $\beta$ -MyHC from the actin filaments, necessary to initiate diastole, and hence, could be considered a loss of function. The controversy also extends to clinical phenotypes as it is debatable whether left ventricular systolic function is increased, as the increased left ventricular ejection fraction would indicate, or decreased, as the results of tissue Doppler studies and magnetic resonance imaging studies would suggest [26, 77]. Impaired myocardial systolic and diastolic function could be one initial functional abnormality that could provide the stimulus for subsequent cardiac hypertrophy and fibrosis because of activation of autocrine and paracrine trophic and mitogenic factors, multiple other initial defects [65]. Other initial defects encompass enhanced myofibrillar  $\text{Ca}^{+2}$  sensitivity and impaired myocardial bio-energetics, as discussed earlier. Collectively, from the above discussion, it could be deduced that a diverse array of primarily abnormalities, probably in combination, contributes to pathogenesis of clinical, morphological, and histological phenotypes of HCM.

#### **GENETIC SCREENING**

Delineation of the molecular genetic basis of HCM during the last 15 years has led to the expectation for routine genetic screening by the patients as well as by the referring physicians. The demand is largely based on the potential utility of genetic testing in identification of individuals and family members who do not carry the causal mutation and thus, are not at the risk of HCM, as well as members who carry the mutation and hence, are at risk [78]. The significance of the latter is further emphasized by the ability

of genetic screening to identify the risk individuals, prior to and independent of the clinical manifestations of HCM. In addition, genetic screening could provide for genetic risk stratification and ultimately, genetic-based interventions to prevent the evolving phenotypes. However, despite the need and in spite of identification of the majority of the causal genes for HCM, routine genetic screening has not been possible and its utility, despite theoretical plausibility, remain to be established. Factors that impede application of the routine genetic screening are diverse and include the diversity of the causal mutations and the low frequency of each specific causal mutation. A desirable genetic screening approach requires high sensitivity and specificity of the screening technique to be performed at a reasonable cost. During the past decade, there have been remarkable advances in the molecular genetic techniques, which have been applied primarily for large-scale and high throughput sequencing and genotyping but not for genetic screening of HCM mutations. The current gold standard technique for mutation detection is direct sequencing of genomic DNA, which has an excellent sensitivity and specificity. However, given the large number of causal mutations, low frequency of each particular mutation, and the absence of dominant mutation hot spots, it would be necessary to sequence all coding regions and exon-intron boundaries of all known causal genes for HCM. Thus, a routine screening would necessitate searching for a single base change in more than 100,000 nucleotides, which at the present time, would be a very tedious task and expensive. Once completed, this approach could lead to mutation detection in ~ 80% of the cases. This situation in HCM is unlike some other single gene disorders, such as cystic fibrosis, wherein a single mutation, namely F508, accounts for approximately 70% of all cases and hence, partial screening is simple and the pre-test likelihood of finding the causal mutation is relatively high.

An alternative approach is to screen the four or five most common genes for HCM, while still costly and labor intensive with the current technology, it could lead to identification of the causal mutations in about 2/3 of all cases. The screening technology is rapidly evolving and the existing techniques, such as specially designed DNA chips or MALDI-TOF, once routinely become available and applied, could provide for routine genetic screening. One could anticipate that within the next few years, advanced mutation screening techniques be applied on a routine basis to screen individuals at risk of developing HCM.

Despite the apparent advantages of genetic screening in identification of those at risk of developing HCM and in prognostications, it is important for the clinicians to be reminded that the clinical utility of genetic testing remain to be established. The best clinical utility of genetic testing at the present time would be in identification of the mutation non-carriers from the mutation carriers, particularly in a family setting. As regards, the impact of genetic factors on prognostication and identification of those at risk of SCD, information not only on the causal genes but also on the modifier genes, will be necessary. More importantly, large-scale clinical studies will be necessary to establish the impact of the specific mutations on clinical outcome, as the current data on clinical impact of genetic mutations is based

on a limited number of studies. While causal mutations are important determinants of clinical phenotypes, information on the modifier genes, which remain largely unrecognized, as well as other factors, such as the environmental factors, would be necessary for the clinical management and counseling of patients with HCM. Thus, clinicians would be able to integrate the genetic and non-genetic factors in counseling and treating the affected individuals and those at risk of HCM.

#### IMPACT OF GENETICS ON TREATMENT OF HCM

There has been no remarkable change in the pharmacological treatment of patients with HCM during the past 2 decades. blockers, calcium channel blockers and less commonly, disopyramide phosphate and amiodarone, the latter for treatment of arrhythmias, have remained the cornerstone of pharmacological treatment of HCM. Unfortunately, current pharmacological treatments are empiric and offer only symptomatic relief and none has been shown to induce regression of cardiac hypertrophy, fibrosis or myocyte disarray, major predictors of mortality and morbidity in HCM [33-36]. Because it is not feasible to correct the underlying genetic defect in HCM with the available technology, the focus of molecular genetics and biology research has been to identify the molecular pathways involved in the pathogenesis of HCM in an attempt to identify novel therapeutic targets. Recent studies in transgenic animal models of HCM, focused on blockade of the intermediary molecular phenotypes, such as blocking signaling kinases involved in induction of hypertrophy and fibrosis, have shown encouraging results in attenuating and reversing the evolving phenotypes [30, 79, 80]. Among the plausible molecular targets are RhoA and Rac1, which are considered essential for induction of cardiac hypertrophy [81, 82]. Activation of RhoA and Rac1 requires membrane association through geranyl geranylation, a process that is blocked by 3-hydroxy-3-methylglutaryl CoA reductase inhibitors [83]. We recently showed treatment with simvastatin attenuated cardiac phenotypes in our transgenic rabbit model, that fully recapitulate the phenotype of human HCM [79]. Accordingly, left ventricular mass was reduced by 37%, septal and posterior wall thickness by approximately 20%, and collagen volume fraction by about 50%, the latter was reduced to normal levels. These histological changes were associated with improvement in left ventricular filling pressure [79]. Several recent studies have corroborated the potential salutary effects of statins in attenuation of hypertrophy and fibrosis in other conditions [84, 85]. Similarly, preliminary studies in humans with heart failure have supported the potential beneficial effects of statins in prevention of heart failure and improvement of cardiac hemodynamic parameters and survival [86-89]. However, whether the findings could extend to human patients with HCM is largely unknown and is under investigation.

Other notable studies in animal models of HCM, with potential applications in human HCM, are the use of spironolactone or losartan to reverse interstitial fibrosis [30, 80]. Because interstitial fibrosis has been associated with arrhythmogenesis and the risk of SCD [33], it is interesting to determine whether normalization of fibrosis would be

associated with the reduced risk of SCD and cardiac arrhythmias in patients with HCM. The potential beneficial effects of blockade of the renin-angiotensin-aldosterone system in treatment of patients with HCM also remain to be tested in human patients.

### INVASIVE THERAPEUTIC INTERVENTIONS IN HCM

Invasive therapeutic interventions are reserved for a small group of patients with HCM who are symptomatic, despite optimal medical therapy, have an inter-ventricular septal thickness of 15 mm or greater and a significant outflow tract gradient obstruction (>50 mmHg at rest). The conventional approach has been surgical resection of a small portion of the thick inter-ventricular septum through a trans-aortic approach (Morrow procedure) [90]. A recently established alternative technique is trans-catheter septal ablation, whereby a small amount of pure ethanol is infused into the main septal perforators of the left anterior descending artery in order to induce local myocardial necrosis and hence, partial ablation of the thick inter-ventricular septum [91]. While there are no randomized clinical studies comparing the clinical outcomes after surgical myectomy and percutaneous transcatheter septal myocardial ablation, retrospective analysis of non-randomized data show an overall equal effectiveness of these techniques in reducing the outflow tract gradient and improving symptoms [92-95]. The peri-procedural mortality of both procedures is relatively low and is approximately 1% in the experienced centers. Surgical myectomy when performed in conjunction with coronary artery bypass surgery has a peri-procedure mortality of 1 to 5%. The long-term follow up data is available only for the surgical septal myectomy, which has been remarkable for an excellent survival and efficacy [94]. The intermediary follow up data for ethanol septal ablation is also promising and is remarkable for an acceptable recurrence rate. One noteworthy complication of the ethanol septal ablation is the relatively high incidence of heart block and conduction defects requiring of permanent pacemaker implantation in approximately 1/4th of the patients [96]. It is also notable that neither surgical nor ethanol septal ablation is considered cure or sufficient to cure the disease. Close monitoring, follow up and treatment of these patients are warranted.

Dual-chamber pacing is another treatment modality, which has been shown to reduce left ventricular outflow tract gradient in symptomatic patients. The principle of dual-chamber pacing is based on modification of the left ventricular excitation pattern leading to dys-synchronous depolarization and hence, relief of the outflow tract obstruction. The poor results of two randomized clinical studies have reduced the overall enthusiasm in the utility of dual-chamber pacing in patients with obstructive HCM [97, 98].

### FUTURE DIRECTIONS

The rapid pace of evolution of the molecular genetic techniques is expected to make routine genetic screening feasible within the foreseeable future. While the initial screening will focus on identification of the causal mutations, comprehensive genetic analysis will also include

modifier genes, epistatic analysis and epigenetic factors. One could also anticipate that advances in molecular genetic diagnosis will lead to re-classification of "un-explained cardiac hypertrophy" and the distinction between true HCM and HCM phenocopy, which is often not possible based on current clinical diagnostic tools. The distinction is important, since the pathogenesis of the two phenotypes differ significantly and because of the prevalence of HCM phenocopy, which could comprise of approximately 10 - 20% of all cases. Accordingly, "un-explained cardiac hypertrophy" could be classified according to the underlying genetic defect of either mutant sarcomeric proteins and hence true HCM, or as a consequence of mutations leading to storage diseases, infiltrative diseases, and even infection.

Insight into the molecular pathogenesis of HCM is expected to provide new molecular targets for interventions to prevent, attenuate and reverse evolving cardiac phenotypes. Notably, the existing experimental data in animal models of human HCM, have established the reversibility of cardiac phenotypes and have paved the way for clinical trials in humans with HCM to test the potential beneficial salutary effects of drugs targeted toward specific pathways involved in the pathogenesis of human HCM [30, 79, 80]. Thus far, two potential targets have emerged, namely, signaling molecules RhoA and Rac 1, which are considered essential for cardiac hypertrophic response [81, 82]; and the renin-angiotensin-aldosterone system, which is involved in cardiac hypertrophy and fibrosis. Given the slow evolution of phenotypes in humans with HCM and given the relatively low prevalence of this disease in the society, collaborations among investigators would be necessary to perform large-scale longitudinal clinical trials in order to test the potential salutary effects of statins and inhibitors of renin-angiotensin-aldosterone system in humans with HCM.

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