MOLECULAR AND CELLULAR BASIS OF ELECTROPHYSIOLOGICAL DISEASES

The molecular genetics of hypertrophic cardiomyopathy: prognostic implications

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Introduction

Hypertrophic cardiomyopathy (HCM) is an inheritable cardiac disorder with heterogeneous clinical features. Sudden cardiac death is a frequent complication, occurring at an annual incidence of 2–3% in young, otherwise healthy individuals with HCM and accounting for nearly 35% of all sudden deaths within this age group[^1^,^2^]. Often, sudden cardiac death is the initial manifestation of HCM, underscoring the need for the precise elucidation of the mechanisms that lead to its occurrence.

Over the past decade, modern molecular techniques have greatly increased the understanding of the pathophysiology underlying HCM. Through collaborative efforts, over 100 mutations in eight genes have been found to be responsible for the disease. All presently known aberrations affect the encoding of sarcomeric proteins, whose modification predisposes the heart to myocyte disarray, fibrosis, hypertrophy, and small vessel disease as well as haemodynamic alterations in diastolic and systolic function. Importantly, the natural history of the disease appears to be influenced by the particular protein affected, as well as by the specific changes within the protein encoded by the mutation. This knowledge has provided greater insight into the vulnerability of the myocardial substrate to mechanisms of sudden death. Knowledge of the genetic abnormalities of HCM and their implications will help the clinician to identify HCM patients at high-risk for sudden death, and to make appropriate recommendations in clinical management and prognosis. This article reviews the current understanding of the genetic mutations in HCM with emphasis on prognosis for sudden and disease-related death.

Pathogenesis

**Disease proteins**

Currently, all genes responsible for familial HCM encode proteins that comprise sarcomeres, the basic contractile units within cardiac myocytes. These disease-related proteins are β-myosin heavy chain (β-MHC), essential and regulatory myosin light chains (MLC-1 and MLC-2), troponin T (cTnT), troponin I (cTnl), α-tropomyosin (α-TM), myosin-binding protein C (MyBP-C), and cardiac actin[^3^–^6^]. The gene(s) of a locus on chromosome 7 that has been linked to a family with HCM and Wolff-Parkinson-White syndrome remains unidentified[^7^].

Sarcomeric proteins together exhibit precise stoichiometry and each possesses a specific, interrelated role in the governing of myocyte contraction and relaxation (Fig. 1). Myosin, the principal component of thick filaments, serves as a molecular motor that directs energy from ATP hydrolysis into the movement of sliding filaments. Each myosin molecule consists of two heavy chains (the β-isoform of which predominates in human ventricles; β-MHC), and two essential (MLC-1) and two regulatory (MLC-2) light chains. β-MHC protein is asymmetric, consisting of a globular head, hinge region, and rod-like tail[^8^]. Within the globular head, ATP hydrolysis and actin binding take place to promote flexion of the head over the tail and sliding across the thin filaments. Most β-MHC mutations have been found within the vicinity of the head and hinge regions. Myosin light chain proteins, which are arranged in tandem near the heavy chain hinge region, probably modulate ATPase activity of the myosin head and provide structural support. Troponin complex proteins and α-tropomyosin comprise with actin proteins the sarcomeric thin filaments. Disease-related mutations have been found in α-tropomyosin and in two subunits of the troponin complex, troponins T (cTnT) and I (cTnl). The troponin complex actively binds α-tropomyosin and operates as a calcium-sensitive switch important in the regulation of actin-myosin interaction and force generation. α-Tropomyosin, a rod-shaped dimer of two

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\(\alpha\)-helices, lies within actin filament grooves and serves as a bridge connecting the actin filaments to the troponin complex. The exact function of MyBP-C remains undetermined. Its C-terminal region simultaneously binds titin, a component of elastic filaments, and myosin, bolstering a structural role. Regulatory functions also are theorized from the presence of several N-terminal sites, whose phosphorylation by cAMP- and calcium-calmodulin dependent protein kinases influences myosin ATPase activity and cross-bridge formation.

Theories of pathogenesis

The putative mechanisms by which the mutations lead to HCM are subject to debate. The recognition that mutations occur in genes related to cardiac contractile performance points to HCM as a disease of the sarcomere, whose structural and/or functional alteration underlies the pathophysiology. Two theories of disease pathogenesis prevail: the poison polypeptide hypothesis and the hypothesis of haploinsufficiency.

The poison polypeptide hypothesis is based on the fact that several genetic abnormalities encode mutant proteins that are probably incorporated with wild-type proteins into the sarcomeres of cardiomyocytes. These polypeptides then may act in a ‘dominant-negative’ manner to alter sarcomeric integrity with consequences of myocyte dysfunction and ‘compensatory’ hypertrophy. In 1988, Bejsovec and Anderson first demonstrated in nematodes that stable polypeptides can arise from myosin missense mutations and be incorporated into sarcomeres, resulting in the impairment of sarcomeric assembly and autosomal dominant paralysis. Subsequent studies of cell cultures harbouring mutant HCM genes have also documented the expression and sarcomeric integration of mutant myosin and other disease proteins, with functional impairments occurring after mutant gene expression. These impairments include decreased actin-myosin interaction, reduced cardiac contractility, and diminished ATP hydrolysis, in addition to impact on sarcomere assembly, as observed by Bejsovec and Anderson.

Several in vivo murine models have further examined mutant HCM gene expression and its effects on cardiomyocyte structure and function. Using homologous recombination techniques, Geisterfer-Lowrance et al. introduced the ARG403GLN mutation into the mouse \(\alpha\)-MHC gene (mice have the \(\alpha\)-isoform of MHC). Mice homozygous for the ARG403GLN mutation died within 7 days of birth. In heterozygous animals, impairment of cardiac relaxation emerged at 5 weeks of age followed by the appearance of myocyte disarray, myocyte hypertrophy, and interstitial fibrosis after 15 weeks of age. Separate electrophysiological investigations of these mice have found heterogeneous ventricular conduction properties, alterations in QRS axis, and frequent arrhythmias. The ARG403GLN \(\beta\)-MHC mutation has also been expressed in mice using transgenic techniques. In one study, these animals exhibited cardiac myofibrillar disarray and biventricular hypertrophy, even though mutant myosin comprised only 1 to 12% of the total ventricular myosin. Similarly, other investigators have observed myocyte disarray, diastolic and...
systolic, dysfunction, and a reduction in the number and size of cardiomyocytes in transgenic mice expressing truncated cTnT proteins\cite{21}. These effects occurred at low levels of mutant protein expression (<5% of total cTnT); higher levels of mutant cTnT were uniformly associated with death within 24 h of birth. The documentation of mutant protein expression and sarcomeric integration in these studies have lent support to the poison polypeptide hypothesis. Of note, the disease manifestations in these models were not unlike those found in humans and occur in the presence of modest amounts of mutant proteins.

Alternatively, some genetic defects predictably introduce frameshifts of the coding sequence that result in premature termination of transcription and the production of truncated proteins. Such defects are termed ‘null alleles’ and commonly are found in mutations of the MyBP-C gene. Resultant quantitative deficiencies or the absence of gene products may alter the stoichiometry of sarcomeric proteins and give rise to disease (i.e. the haploinsufficiency hypothesis). The theoretical basis for the role of haploinsufficiency was demonstrated in Drosophila experiments. Drosophila, which were heterozygous for either actin or myosin null alleles, had myocyte disarray and altered myofibrillar turnover\cite{22}. In Drosophila which were double heterozygotes (i.e. the presence of an actin and a myosin null allele), the maintenance of actin–myosin stoichiometry led to the development of myocytes with nearly normal function. Separate studies of Drosophila harboring cTnT mutations also found the rapid degradation of non-assembled, cytosolic mutant cTnT protein\cite{23}. Contractility and relaxation abnormalities also have been reported in mice heterozygous for α-MHC null alleles\cite{24}.

The premise of haploinsufficiency has been challenged, however, because the absence of mutant protein expression and incorporation has not been found in other disease models of HCM. Many of these studies include the aforementioned in vitro and murine investigations. Additionally, successful assimilation of mutant proteins occurs in humans with HCM\cite{12,14}. Of note, in a study of mutant human cTnT and cultured quail myotubes, Watkins et al. specifically addressed the haploinsufficiency hypothesis\cite{15}. These cells were transfected with vectors containing mutant cTnT to ensure near-complete replacement of native cTnT, creating myotubes essentially homozygous for the mutant variant. In contrast to the experiments with Drosophila, sarcomeric expression and incorporation of mutant cTnT occurred, with orderly sarcomeric assembly present in ~86% of these cells. Furthermore, the expression of human mutant, but not wild-type, cTnT resulted in reduced contractile force.

Presently, the impetus for the development of hypertrophy in persons with altered sarcomeric proteins is unknown. The predominant theory is that the observed hypertrophy is part of a compensatory process in the presence of abnormal cellular architecture and/or performance. Several observations support this belief. In vitro cell culture studies have revealed impairment of myocyte contractility following the expression of mutant sarcomeric genes. Decreased maximal shortening velocity, isometric force generation, and calcium-activated contraction force have been documented in cells possessing a number (≥10) of different β-MHC mutations as well as mutations of the cTnT disease gene\cite{12-15,25}. In studies of Dicyostelium discoideum, in which site-directed mutagenesis of the myosin II gene resulted in six mutations found in humans, mutant myosins displayed reduced force generation, actin affinity, and ATPase activity\cite{26}. The functional impairments of each mutation were also related to their previously reported prognostic features. Slow skeletal muscle fibres taken from patients possessing the GLY741ARG or ARG403GLN β-MHC mutations also show evidence of diminished contractile properties\cite{12}. Of note, with few exceptions, the myocyte hypocontractility observed in many of these investigations has been observed in the absence of structural derangement, suggesting that functional deterioration may precede the structural anomalies in hypertrophic cardiomyopathy.

Nonetheless, current investigations of the consequences of mutant proteins have certain limitations. In vitro motility studies are conducted in the absence of in vivo loading conditions, which may affect myocyte response in measures of contractility and also be responsible for structural defects. Moreover, several investigators have reported heightened filament sliding rates, force generation, and calcium sensitivity following mutant sarcomeric gene expression, questioning the role of a compensatory impetus for myocyte hypertrophy\cite{27,28}. Enhanced motility perhaps might explain the less marked ventricular hypertrophy seen in certain individuals. In addition, current in vivo models of familial HCM are heterologous systems in which mutant human β-MHC or cTnT genes are introduced into mice. Techniques used for this introduction may lead to artefacts: homologous recombination may result in the removal of a pertinent wild-type region(s); transgenic techniques may generate unnatural numbers of alleles. Importantly, though murine models have disease morphology resembling that of human HCM, the precise mechanisms by which the genetic mutations usher in the histological and gross functional abnormalities of HCM remain to be delineated in vivo. The hypothesis of compensatory hypertrophy does not explain several distinguishing features of HCM, such as myocyte disarray, small vessel disease, interstitial and replacement fibrosis, relaxation abnormalities, and supranormal indices of systolic performance.

Multiple mechanisms of disease are likely to be operative due to the heterogeneous nature of HCM. In families in which affected individuals possess the same disease mutation, there is often a range of morphological and clinical features, suggesting that other genetic and non-genetic factors influence the disease process. Studies of gene polymorphisms with the renin-angiotensin-aldosterone system (RAAS) have supported the role of modifying genes in the morphological...
expression of HCM[51–54]. The fact that all current disease genes encode sarcomeric components suggests that the alteration in sarcomeric structure and/or function is a unifying common pathway in the pathogenesis of HCM. Presently, known disease genes account for ~50% of cases of HCM. Further recognition of other disease-related genes and the understanding of their functional consequences are still required.

**Prognostic implications of disease genes**

HCM is a disease entity that results from mutations in at least eight sarcomeric genes. These genes display heterogeneity in their expression in relation to penetrance and prognosis. Penetrance is defined as the proportion of individuals with a genetic mutation who have disease expression of that mutation. Commonly reported endpoints for prognostic evaluations in HCM are sudden death and other cardiovascular-related causes, such as congestive heart failure and cerebrovascular accidents.

**B-myosin heavy chain (β-MHC)**

The molecular basis for HCM was first demonstrated through the identification of disease-related β-MHC mutations on chromosome 14[35]. Over 50 mutations within the β-MHC gene have been found to be responsible for the disease, and these may account for 30% of all persons with HCM[36]. Genotype–phenotype correlation studies in HCM have been performed most extensively for the β-MHC mutations. Characteristically, β-MHC mutation-related HCM is markedly heterogeneous with respect to morphological expression and the natural history of the disease in afflicted individuals.

β-MHC mutations in HCM have been classified as being of low, intermediate, or high risk, in relation to the occurrence of sudden death and disease-related death (Table 1). Presently, the ARG403GLN, ARG719TRP, ARG453CYS mutations are the β-MHC mutations most often associated with an adverse prognosis. The ARG403GLN mutation, one of the most widely reported HCM-related mutations, typically exhibits high penetrance and a marked risk of premature sudden death. In a combined analysis of the three largest investigations of ARG403GLN mutation-related HCM, 23 sudden deaths and 15 additional disease-related deaths occurred in 81 individuals[37–39]. The mean age of sudden death was 33 years, leading to a cumulative survival rate of ~50% by 40 years of age. In the two studies in which penetrance was reported, disease manifested in 88 to 100% of adults. Such high penetrance is highlighted further by the study of an Italian family (n=7), in which all children (n=4, age range, 2–7 years) exhibited phenotypic manifestations and a mean maximal left ventricular wall thickness of 21·5 mm (range, 15–27)[40]. HCM-related mortality rates in persons with ARG719TRP and ARG453CYS mutations are comparable to those with the ARG403GLN mutation. In 61 individuals with the ARG719TRP mutation, 35 died of disease-related death, of which 22 were sudden[41,42]. The resultant life expectancy of persons with this mutation was 38 years. Similarly, in 26 individuals with the ARG453CYS mutation, 14 disease-related deaths and eight sudden deaths were observed, occurring most commonly in the third decade of life[37]. The premature death rates of HCM patients with the ARG403GLN, ARG719TRP, and ARG453CYS β-MHC mutations are substantially greater than the overall mortality rate of the entire HCM population (1–2% per year)[43], emphasizing the highly malignant nature of these β-MHC mutations.

Near-normal life expectancy distinguishes the disease manifestations of the VAL606MET, LEU908VAL, and GLY256GLU β-MHC mutations. The VAL606MET mutation was associated with only one premature sudden death (age=13 years) and one disease-related death (age=17 years) out of 32 individuals in two large investigations[37,39]. Similarly, in 46 persons affected with the LEU908VAL mutation[38] and 39 individuals with the GLY256GLU mutation[40], only three premature sudden deaths were observed. Diminished familial

<table>
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<th>β-MHC mutation</th>
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qΔ=the overall change in amino acid residue conferred by the mutation; DRD=disease-related death, as determined by the investigators of the studies; SD=sudden death.
penetrance also characterizes the LEU908VAL and GLY256GLU mutations, with a normal echocardiogram being present in 37% and 44% of adults, respectively. It has been suggested that low disease penetrance and less severe hypertrophy may relate to a low risk of sudden death in β-MHC mutations. The survival rates of persons with the VAL606MET, LEU908VAL, and GLY256GLN β-MHC mutations all surpass 90% at 60 years of age, closely paralleling that of the age-matched individuals within the general population.

The ARG249GLN and GLU930LYS β-MHC mutations confer an intermediate prognosis in HCM. In an investigation of 24 individuals with ARG249GLN mutation-related HCM, 10 disease-related deaths and four sudden deaths were observed. Although the number of deaths due to HCM was high, log-rank analyses for persons with the ARG249GLN mutation demonstrated survival rates greater than those with the ARG403GLN (P=0.027 vs ARG249GLN) and the ARG403GLN (P=0.015 vs ARG249GLN) mutations, nearly approaching that of individuals with the VAL606MET β-MHC mutation (P=0.067 vs ARG249GLN). The better survival result of these patients, nonetheless, may be due only to a shift in the mean age of death of these patients, which was 49 ± 2 years and substantially later than the mean age of death for other adverse mutations. In a second study of 10 individuals with the ARG249GLN mutation, no sudden deaths or disease-related deaths were observed. Only one individual, however, was older than 49 years in this report, pointing to the possibility that disease manifests later in patients with this particular β-MHC mutation. In persons with the GLY930LYS mutation, two premature sudden deaths out of 16 affected individuals have been reported in a single study. One member of this family also underwent transplantation for progressive heart failure.

The vast majority of disease-related β-MHC mutations are missense alterations that result in single amino acid substitution. It has been proposed that the ‘degree of malignancy’ of the β-MHC mutation relates to the change in residue charge imparted by amino acid substitution. Theoretically, because amino acids differ in terms of structure and side chain charge or polarity, the substitution of an amino acid may lead to the destabilization of the protein structure and function. This effect may be even more emphasized if the substitution occurs in critical sites, such as the areas involved in ATP hydrolysis and in interactions with thin filaments, leading to the production of a myocardial substrate more vulnerable to mechanisms of sudden death.

In 1992, Watkins and colleagues first demonstrated the relationship between β-MHC amino acid substitution and the natural history of the disease by reporting survival rates for conservative (VAL606MET) and non-conservative (ARG403GLN, ARG453GLY, and ARG249GLN) β-MHC mutations. Each of these non-conservative mutations involves a negative charge change in the substituted amino acid residue (−1), in contrast to the VAL606MET mutation which results in no overall change in charge. Survival analysis of individuals with each of these mutations demonstrated markedly diminished life expectancies for those with non-conservative mutations compared to those with the VAL606MET mutation.

The probable importance of specific changes in residue properties with respect to prognosis for β-MHC-related HCM is further bolstered by studies of different residue substitutions in hot spot mutation areas, particularly at codon 403. Independently occurring mutations at this codon have been described in a number of families worldwide (the ARG403GLN, ARG403TRP, and ARG403GLU β-MHC mutations). In 32 family members afflicted with HCM and the ARG403TRP β-MHC mutation, low adult penetrance (25% with maximal left ventricular wall thickness <13 mm) and no familial history of sudden death were observed. In 22 HCM patients with the ARG403LEU β-MHC mutation, three sudden deaths and an additional five disease-related deaths had occurred. Adult penetrance was 66% and the mean age of death was 38 years (range, 14–65). Therefore, although the ARG403TRP, ARG403GLN, and ARG403LEU β-MHC mutations all affect the same codon in the β-MHC gene, prognosis and penetrance appears to vary across each specific mutation, with a significantly better outcome portended by the ARG403TRP mutation. The
cumulative survival rate at 50 years of age for those with the ARG403LEU mutation was 42%, which is similar to that reported for the ARG403GLN mutation, but is in direct contrast to the rate of 100% in those individuals with the ARG403TRP mutation. Other independent investigators also have pointed to the impact of residue change on prognosis. The PHE513CYS and the aforementioned LEU908VAL β-MHC mutations are conservative amino acid substitutions with reported low prevalence of sudden death[38,41].

The adverse prognosis of certain β-MHC mutations, however, cannot be explained solely by different amino acid properties. The GLY256GLN β-MHC mutation indicates a positive change in residue change (+1), but is associated with a benign prognosis[44]. This observation suggests that mutations in non-critical regions of the β-MHC protein, irrespective of the specific residue substitution, may not have significant effects on β-MHC protein structure and/or function. Substantial heterogeneity in disease manifestation also occurs within HCM families, despite the presence of the same β-MHC mutation in all affected members. A number of persons with a particular β-MHC mutation survive to normal or near-normal life expectancy in all reports, suggesting the presence of certain genetic and/or environmental modifying factors that affect prognosis. The influence of these factors has been raised by small, conflicting reports of genotype — phenotype correlation studies of β-MHC mutations. In a series of six HCM patients of Korean ancestry with the ARG403GLN β-MHC mutation, no history of sudden death was discovered[49]. These same authors also presented a series of 8 VAL606MET β-MHC mutation-related HCM cases, in which four premature sudden deaths had occurred. Further studies are still required to reveal the roles of other genetic and non-genetic factors in HCM.

Cardiac troponin T (TnT)

In 1994, the cTnT gene on chromosome 1 was recognized as a disease-related gene in HCM[49]. Twelve mutations subsequently have been identified within the cTnT gene, accounting for approximately 15% of all cases of HCM[36,50–53]. With rare exception, the distinguishing features of disease due to cTnT mutations are mild ventricular hypertrophy and a poor prognosis that is markedly dissociated from the extent of hypertrophy. Studies of cTnT mutation-related HCM have demonstrated a risk of sudden death that is equivalent or worse than the risk found in the most adverse β-MHC mutations (Fig. 3). In the initial natural history description of cTnT mutations, 39 sudden deaths and an additional 11 disease-related deaths were observed in 112 individuals[50]. Mean maximal left ventricular wall thickness (MLVWT) was 16.7 ± 5.5 mm, which is substantially less than that reported for β-MHC mutations (23.7 ± 7.7 mm). Moreover, 25% of those older than 16 years had normal echocardiograms (MLVWT<13 mm). In contrast to β-MHC mutation-related HCM, the occurrence of sudden death did not differ significantly among cTnT mutations for which sufficient numbers enabled survival analyses (Table 2).

Other independent investigators have also found a marked risk for sudden death due to cTnT mutations. Six sudden deaths, the mean age of occurrence of which was 17 ± 9 years, have been described in 19 individuals with the ARG92TRP cTnT mutation[50]. Adult penetrance was 40% by echocardiography and 80% with the addition of electrocardiography. This observation is consistent with the relatively lower penetrance rates of cTnT mutations in contrast to that of other HCM disease mutations (Fig. 4). Furthermore, as reported in other studies, most deaths in this investigation had occurred by the third decade: the cumulative survival rate was 34% by 28 years of age. In a study of the ALA104VAL cTnT mutation, two sudden deaths (ages 36 and 50 years) out of four individuals were detected. Mean MLVWT in these individuals was 17.2 mm (range, 15 to 20 mm). An additional three other sudden deaths (ages 15, 44, and 48 years) without disease documentation in this family also were probably due to HCM.

A recent investigation has raised the possibility of the existence of benign cTnT mutations in HCM[55]. In a report of the PHE110L/LE cTnT mutation, only two sudden deaths and two additional disease-related deaths
in 16 afflicted individuals were found. Although the phenotypic expression was similar to that of other cTnT mutations (mean MLVWT=17·3±5·8 mm), the cumulative survival rate was approximately 80% at 60 years of age, near the rate found in the benign -MHC mutations. Nonetheless, the interpretation of this study requires the recognition that prognostic data for this mutation was pooled from six different families, each of whom contributed a small number of probands. Importantly, the number of persons for whom disease status and the cause of death were not determined (n=28) outweighed the number of persons for whom disease was confirmed (n=27). As independent corroboration of this study is required, it is generally agreed that the vast majority of cTnT mutations in HCM are associated with an adverse risk of sudden death.

Myosin-binding protein C (MyBP-C)

In 1993, Carrier and colleagues first linked the locus containing the MyBP-C gene on chromosome 11 to HCM[54]. Shortly thereafter, Bonne[55] and Watkins[56] simultaneously identified specific mutations within the MyBP-C gene and 22 defects now have been recognized. Collectively, MyBP-C mutations comprise about 15% of all cases of HCM[57]. Initial reports of MyBP-C mutations in HCM suggested that these genetic defects were benign due to the relatively low frequency of disease-related deaths observed in proband families[56]. However, more recent, larger studies have found disease due to MyBP-C mutations to have age-dependent penetrance and a high risk of sudden death once expression of the disease occurs. In the description of 212 individuals with these genetic defects, 34 sudden deaths were noted[57]. Although this incidence approaches that of other high risk mutations in HCM, the cumulative survival rate at 50 years of age approached 70%, which is much greater than that of disease due to cTnT and the most adverse -MHC mutations. This enhanced early survival reflects the late penetrance of the MyBP-C mutations that featured prominently in these cases. In contrast to the cTnT and -MHC mutations, which displayed nearly complete penetrance by the second or third decade of life, phenotypic expression of MyBP-C mutations remained diminished until the fifth decade (Fig. 5). Less than two-thirds of persons younger than 40 years of age had cardiac hypertrophy by conventional criteria.

![Figure 4: Penetrance studies of HCM mutations. Bar graphs represent means of reported penetrance values of corresponding HCM mutations in adults (>16 years, except for MyBP-C study in which adults were defined as ≥18 years). Penetrance by echocardiography was defined as MLVWT >13 mm. Penetrance by electrocardiography was defined as evidence of left ventricular hypertrophy (Romhilt-Estes score or Sokolov voltage criteria) as assessed by the study authors. Although the inclusion of minor electrocardiographic abnormalities would probably have augmented the penetrance rates in this figure, the definition of these abnormalities varied among published reports. Only published data were used for this figure[38,39,46,50,51,58,59,61]. □=ECG; ◻=Echo; □=both.](image-url)
Overall, the risk profile in patients with MyBP-C mutations resembles that of the most adverse β-MHC mutations, with an upward shift in the age of disease manifestation. Apart from this discrepancy, patients with MyBP-C mutations share many morphological and clinical features with those who have disease due to β-MyHC mutations[48,58]. The characterization of the phenotypic manifestations of MyBP-C mutations underscores the need for continued screening of elderly persons who may be affected with HCM. These individuals, though having survived late into life, may still be at marked risk for sudden death.

\[\text{α-Tropomyosin}\]

Mutations in the gene encoding α-tropomyosin on chromosome 15 were first linked to HCM in 1994[59]. Four missense mutations have now been described that comprise 3–5% of all cases of HCM[37,60]. Characteristic features of α-tropomyosin disease are heterogeneous ventricular hypertrophy and a moderate prognosis with regards to sudden death and other disease-related deaths.

In a study of 21 persons from three families with the ASP175ASN α-tropomyosin mutation, full adult penetrance and a range of ventricular hypertrophy similar to that found in β-MHC mutation disease were observed[61]. Of note, either mild (MLVWT = 15±2.7 mm), moderate (MLVWT = 18±2.1 mm), or marked (MLVWT = 24±4.5 mm) ventricular hypertrophy characterized the phenotype of each family (DB, MI, and DT, respectively). In fact, eight members of family DT had a MLVWT of 28 mm, pointing to the likely role of other genetic and/or local modifying factors. Also, evidence for mutation-specific hypertrophy exists in α-tropomyosin disease. In another study of 13 persons with the GLU180GLY and eight persons with the ASP175ASN α-tropomyosin mutation, mean MLVWT was 12.5±4.7 mm (range 8 to 23 mm) and 30.3±3.1 mm, respectively[59]. Penetrance of the GLU180GLY by echocardiography was 38%, which is less than the 100% penetrance reported for the ASP175ASN mutation in this study and other reports. Despite dissimilar degrees of hypertrophy, disease-related survival in individuals with α-tropomyosin mutations parallels that of persons with less severe β-MHC mutations, as only a few premature sudden deaths (3) have been noted in genotype–phenotype analyses. Moreover, a number of persons may have lower morbidity, with nearly 85% of those afflicted with the ASP175ASN α-tropomyosin mutation having minimal or no symptoms of disease[61]. An exception is a small series of HCM due to the ALA63VAL (n=2), LYS70THR (n=3), and ASP175ASN (n=3) α-tropomyosin mutations, that has reported an unusually high incidence of cardiac dilatation and deaths due to congestive heart failure[60]. Nonetheless, current investigations support a benign prognosis for

\[\text{Molecular genetics of hypertrophic cardiomyopathy 11}\]
α-tropomyosin disease, with a near-normal life expectancy in most cases of these genetic defects.

**Myosin light chain proteins (MLC), Troponin I (cTnl), and Actin**

Mutations in the genes for essential and regulatory myosin light chain proteins (MLC-1 and MLC-2), actin, and cTnl collectively comprise about 5% of all cases of HCM. The MLC-1 and MLC-2 proteins are encoded by genes on chromosome 3 and 12, respectively. Early reports suggested an association between mid-cavity obstruction and MLC-1 and MLC-2 mutations. Independent confirmation of this genotype–phenotype correlation is still required, however, as other investigations have not made this observation. The actin gene is on chromosome 15 and the cTnl gene on chromosome 19 have only been recently identified to cause HCM. Presently, genotype–phenotype correlations of MLC-1, MLC-2, cTnl, and actin mutations with respect to sudden death and/or disease-related death are largely unknown, as their rarity has been prohibitive of large analyses.

**Limitations**

Much insight has been gained from genotype–phenotype analyses, yet several important inherent limitations of these studies exist. Few or single kindreds frequently comprise the study groups, and mathematical assumption limit survival analyses to families of adequate size. Data therefore may not be universally applicable. Investigations are also being performed mainly at tertiary centres, where referral bias may artificially elevate the death rates of the study populations and confound data interpretation. Furthermore, the analyses of the current investigations are commonly retrospective and do not take treatment into account in the assessment of outcome. Kindred data can be gleaned only from those individuals for whom survival or cause of death can be accurately determined. This problem may be important, particularly in persons with hypertrophic cardiomyopathy, where deaths most commonly occur in the young and may not be completely investigated. Additionally, because the prognosis of mutations may be age-dependent, analyses at certain points in time may not accurately reflect the risk of sudden death.

**Future considerations**

The recognition of persons with HCM who are at increased risk for sudden death remains an important clinical challenge, particularly because of the heterogeneous features of the disease. From genotype–phenotype correlation studies, however, clear patterns of disease are rapidly emerging, providing opportunities for appropriate medical intervention. Genetic mutations in HCM carry prognostic significance, and routine genotype determination will be of significant benefit in the precise identification of high-risk individuals. Currently, genotype analysis remains within the confines of research institutions, as the necessary techniques still require considerable time, financial support, and technical expertise. As genetic testing becomes more widely available, the clinician and patient alike stand to benefit, not only in the routine clinical management of HCM, but also in the preclinical diagnosis of persons at risk.

**References**


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