Multiple Mutations in Genetic Cardiovascular Disease

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Over the last 2 decades, major advances have been made in our identification and understanding of the genetic basis of cardiovascular disease. More than 40 cardiovascular disorders have now been identified to be directly caused by single-gene defects. These disorders span all aspects of cardiovascular disease and affect all parts of the heart structure. They include the inherited cardiomyopathies such as hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy, and arrhythmogenic right ventricular dysplasia; primary arrhythmogenic disorders such as familial long-QT syndrome (LQTS) and Brugada syndrome; congenital heart diseases such as Marfan syndrome; and metabolic disorders such as familial hypercholesterolemia (FH). Until recently, these cardiac genetic disorders have been thought to involve only single-gene defects (ie, in an individual patient, 1 mutation in a single gene leads to a disease).

A common feature of almost all genetic cardiovascular diseases is the clinical or phenotype heterogeneity observed in the affected individuals both within and between families. Despite harboring the same gene mutation, affected individuals (eg, siblings) can often have marked clinical variability, ranging from no symptoms to severe heart failure and premature death. This widespread clinical heterogeneity suggests other factors apart from the gene mutation itself are important in modifying the clinical phenotype, either by exacerbating or protecting against the disease.

These modifying factors are poorly understood and may include a number of factors (Figure 1). These include environmental factors such as exercise and diet, age and gender-related influences, and secondary genetic factors. To date, the role of secondary genetic factors has focused largely on gene variants or polymorphisms that do not directly cause disease but may influence regulatory factors such as gene promoter regions altering gene expression or influence the function of key enzymes important in normal cardiovascular biology. An example is the insertion/deletion polymorphism of the angiotensin-converting enzyme gene, which has been implicated as a modifying factor in a number of aspects of cardiovascular disease, including extent of cardiac hypertrophy, progression to heart failure, and sudden death (for review, see reference 3). However, most recently, there is an emerging recognition that a proportion of patients carry 2 (multiple) independent disease-causing gene mutations (ie, not polymorphisms), leading to more severe clinical disease. These mutations can occur in the same gene (compound mutation) or in 2 different genes (double mutation), as indicated in Figure 2. This challenges the well-accepted paradigm in autosomal dominant monogenic medical diseases that 1 mutation in a single gene is the direct cause of disease and has major implications on the clinical evaluation, diagnosis, and management of families with a genetic cardiovascular disease.

This review will focus on the role of multiple mutations in patients with genetic cardiovascular disease. Specifically, HCM will be the focus of this review, given that it is the disease most thoroughly studied in terms of both genetic causes and clinical outcomes. The impact of multiple mutations both in our understanding of underlying pathogenic mechanisms, as well as in diagnosis and counseling in families with genetic heart disease will also be discussed.

Multiple Mutations in HCM

Of all the inherited cardiovascular disorders, HCM was the first in which a genetic basis was identified and, as such, has acted as a paradigm in terms of how to study a genetic cardiovascular disease. Although major advances have been made in understanding the genetic causes of HCM, recent reports suggest a significant proportion of patients with HCM harbor multiple mutations.

Clinical Basis of HCM

HCM remains the most common cardiovascular genetic disorder, occurring in at least 1 in 500 people in the general population. HCM is a primary inherited disorder of the myocardium characterized by hypertrophy, usually of the left ventricle, in the absence of other loading conditions such as hypertension. Individuals with HCM exhibit marked diversity in their morphological features and clinical manifestations, ranging from no symptoms to heart failure and sudden death. This clinical heterogeneity reflects the complex pathophysiology underlying the disorder, which includes not only...
diastolic dysfunction, but also arrhythmogenic substrates leading to ventricular arrhythmias, small-vessel disease leading to subendocardial ischemia, and left ventricular outflow tract obstruction.9,10 Therefore, HCM is a disease with a multitude of potential cardiac pathologies, resulting in a diverse range of clinical outcomes.

Although relatively uncommon, sudden cardiac death remains the most devastating complication of HCM. The prevalence of sudden death ranges from 0.5% to 5% in various reported studies, most of which are derived from tertiary referral centers and therefore have an inherent tertiary referral bias of more severe cases.10,11 Sudden cardiac death in HCM is often associated with exercise and, in some cases, related to high-level, high-profile competitive sports.11,12 Over recent years, a number of risk stratification factors have been identified in patients with HCM. Specifically, a positive family history of HCM, a previous resuscitated cardiac arrest, a left ventricular wall thickness greater than 30 mm, syncope, and nonsustained ventricular tachycardia on 24-hour ambulatory ECG monitoring are all considered important risk factors for sudden death in HCM.13 Other factors associated with an increased risk of sudden death in HCM include an abnormal blood pressure response to exercise, significant left ventricular outflow tract obstruction, and the presence of specific “malignant” gene mutations.14,15

Although important advances have been made in identifying factors that place patients with HCM at highest risk of developing the 2 most serious complications of disease (ie, progressive heart failure and sudden cardiac death), there are likely to be other key factors that may help to stratify risk more precisely.

Genetic Basis of HCM
Since 1990, more than 400 mutations in at least 13 genes have been identified in patients with HCM (summarized in Table 1).9,16 Approximately 70% of all mutations are identified in the 2 most common genes: β-myosin heavy chain (βMHC) and myosin-binding protein C (MyBP-C).5 All mutations are inherited in an autosomal dominant pattern and occur in genes that encode proteins of the sarcomere or are associated with sarcomere-related structures. As with the clinical variability of disease, genetic heterogeneity is an important characteristic of HCM. Many other disease genes have been implicated in HCM, but these more likely represent diseases which clinically mimic HCM but actually represent a different pathological disease, eg, glycogen storage diseases caused by mutations in the PRKAG2 gene.17,18

Multiple Mutations in HCM Patients
Most recently, a number of genetic studies in HCM have suggested that up to 5% of families carry 2 distinct disease-causing gene mutations.5–6 Compared with individuals with single-gene mutations, HCM patients with homozygous and double or compound heterozygous mutations typically present with more severe left ventricular hypertrophy and a higher incidence of sudden cardiac death events, including resuscitated cardiac arrest, among family members. Collectively, patients with multiple mutations are also significantly

Table 1. Genetic Basis of HCM

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Encoded Protein</th>
<th>Disease %</th>
</tr>
</thead>
<tbody>
<tr>
<td>βMHC</td>
<td>β-Myosin heavy chain</td>
<td>30 to 35</td>
</tr>
<tr>
<td>MyBP-C</td>
<td>Myosin binding protein C</td>
<td>20 to 30</td>
</tr>
<tr>
<td>cTnT</td>
<td>Cardiac troponin T</td>
<td>10 to 15</td>
</tr>
<tr>
<td>αTMI</td>
<td>α-Tropomyosin</td>
<td>5 to 15</td>
</tr>
<tr>
<td>cTnl</td>
<td>Cardiac troponin I</td>
<td>&lt;5</td>
</tr>
<tr>
<td>CSRP3</td>
<td>Cardiac muscle LIM protein</td>
<td>&lt;5</td>
</tr>
<tr>
<td>TCAP</td>
<td>Telohelin</td>
<td>&lt;2</td>
</tr>
<tr>
<td>MYL2</td>
<td>Regulatory light chain</td>
<td>&lt;1</td>
</tr>
<tr>
<td>MYL3</td>
<td>Essential light chain</td>
<td>&lt;1</td>
</tr>
<tr>
<td>ACTC</td>
<td>Actin</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>TTN</td>
<td>Titin</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>αMHC</td>
<td>α-Myosin heavy chain</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>cTnC</td>
<td>Cardiac troponin C</td>
<td>&lt;0.5</td>
</tr>
</tbody>
</table>

Figure 1. Clinical and genetic heterogeneity in genetic cardiovascular disease. A number of potential modifying factors are likely to be involved in disease pathogenesis.

Figure 2. Zygosity of disease-causing mutations. Individuals carrying a single disease-causing mutation are heterozygous at that allele, whereas individuals carrying multiple disease-causing mutations can be trans or cis compound heterozygous or homozygous if the mutations are in the same allele, or double heterozygous if the mutations are in different alleles. Crosses indicate disease-causing mutations; boxes, alleles.

Heterozygous

Compound Heterozygous (trans)

Compound Heterozygous (cis)

Homozygous

Double Heterozygous
Four additional homozygous mutations in MyBP-C had left ventricular hypertrophy, both were asymptomatic. Heterozygous members showed mild HCM. However, each family had a homozygous child who died of heart failure in the first year of life. These observations support the notion of a mutation "dosage effect," whereby a larger amount of defective protein leads to greater disruption to normal sarcomere function and resulting in a more severe clinical course.

Homozygous HCM Mutations
A number of HCM cases have been reported over the last decade in which homozygous mutations have been identified, most frequently associated with both more severe clinical disease and, in some cases, presentation in childhood (Table 2). Nishi et al23 first reported 2 brothers homozygous for the Lys935Glu mutation in the βMHC gene. They presented with severe left ventricular hypertrophy and a clinical course culminating in severe progressive heart failure and sudden death in their fourth decade of life. Both parents were heterozygous for the Lys935Glu mutation, and although both had left ventricular hypertrophy, both were asymptomatic. Four additional homozygous mutations in βMHC have since been reported, all demonstrating a severe clinical course resulting in a dilated form of cardiomyopathy, associated with heart failure, sudden death, and heart transplantation.5,19–22,29 Homozygous mutations have also been described in the MyBP-C gene in patients with HCM. Although single-gene mutations in MyBP-C tend to cause later onset and less severe disease, individuals homozygous for Arg810His and Pro873His substitutions have more severe disease than heterozygous individuals.28 Furthermore, whereas HCM presenting in early childhood is relatively uncommon, 2 homozygous mutations that severely truncate the MyBP-C protein have been reported as causing neonatal HCM, resulting in sudden cardiac death within the first year of life. In 1 report, a single mutation segregated in 3 Amish families with heterozygous members showing mild HCM. However, each family had a homozygous child who died of heart failure in the first year of life. The intronic mutation identified was shown to cause skipping of exon 30, leading to a premature translation stop codon 211 amino acid residues from the carboxyl-terminal end, thus removing the major myosin-binding domain of MyBP-C.

Fewer homozygous HCM cases have been reported in the less commonly associated HCM genes. Homozygous Phe110Ile and Ser179Phe mutations in the cardiac troponin T (cTnT) gene have been reported in 2 large HCM families, associated with significant biventricular hypertrophy and a high incidence of sudden death.27,28 Each mutation was not fully penetrant as some heterozygous family members were asymptomatic, whereas others had mild left ventricular hypertrophy. The left ventricular septal wall thickness for 2 homozygous Phe110Ile patients was greater than family members heterozygous for this mutation, suggesting that 1 normal cTnT allele can largely compensate for the disruption caused by the Phe110Ile allele.

Collectively, individuals and families with homozygous HCM mutations have been described. Their clinical profile is highlighted by earlier onset of disease, more significant cardiac hypertrophy, and more frequent progression to heart failure and sudden cardiac death events when compared with family relatives who carry single heterozygous mutations. These observations support the notion of a mutation "dosage effect," whereby a larger amount of defective protein leads to greater disruption to normal sarcomere function and resulting in a more severe clinical course.

Double Heterozygous HCM Mutations
Double heterozygous mutations, whereby single disease-causing mutations in 2 different genes exist, have been reported in several HCM families (Table 3). Analogous to families with homozygous mutations, double heterozygous HCM patients also collectively display more severe clinical disease. In the first such report, in a family in which both the Glu1096Ter nonsense mutation in MyBP-C and the Glu483Lys mutation in the βMHC genes coexisted, affected family members who carried both mutations had more severe left ventricular hypertrophy compared with those who carried only 1 of the 2 mutations, suggesting an additive effect.5,30

Table 2. Homozygous HCM Mutations

<table>
<thead>
<tr>
<th>Gene 1</th>
<th>Mutation 1</th>
<th>Gene 2</th>
<th>Mutation 2</th>
<th>SD/HTx</th>
<th>IVS, mm (Age, y)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>βMHC</td>
<td>Lys207Gln</td>
<td>βMHC</td>
<td>Lys207Gln</td>
<td>No</td>
<td>21 (64)</td>
<td>Mohiddin et al19, Alpert et al20</td>
</tr>
<tr>
<td>βMHC</td>
<td>Arg403Trp</td>
<td>βMHC</td>
<td>Arg403Trp</td>
<td>HTx</td>
<td>20 (38)</td>
<td>Keller et al21</td>
</tr>
<tr>
<td>βMHC</td>
<td>Asp778Glu</td>
<td>βMHC</td>
<td>Asp778Glu</td>
<td>SD</td>
<td>19 (NA)</td>
<td>Richard et al22</td>
</tr>
<tr>
<td>βMHC</td>
<td>Arg869Gly</td>
<td>βMHC</td>
<td>Arg869Gly</td>
<td>No</td>
<td>35 (29)</td>
<td>Richard et al22, Richard et al6</td>
</tr>
<tr>
<td>βMHC</td>
<td>Glu335Lys</td>
<td>βMHC</td>
<td>Glu935Lys</td>
<td>No</td>
<td>13 (25)</td>
<td>Nishi et al23</td>
</tr>
<tr>
<td>MyBP-C</td>
<td>Gln767Ter</td>
<td>MyBP-C</td>
<td>Gln767Ter</td>
<td>No</td>
<td>16 (&lt;1)</td>
<td>Richard et al25</td>
</tr>
<tr>
<td>MyBP-C</td>
<td>Ala627Val</td>
<td>MyBP-C</td>
<td>Ala627Val</td>
<td>No</td>
<td>28 (47)</td>
<td>Garcia-Castro et al24</td>
</tr>
<tr>
<td>MyBP-C</td>
<td>Arg810His</td>
<td>MyBP-C</td>
<td>Arg810His</td>
<td>No</td>
<td>32 (32)</td>
<td>Nanni et al25</td>
</tr>
<tr>
<td>MyBP-C</td>
<td>Pro873His</td>
<td>MyBP-C</td>
<td>Pro873His</td>
<td>No</td>
<td>27 (27)</td>
<td>Nanni et al25</td>
</tr>
<tr>
<td>MyBP-C</td>
<td>Asp1064fsX38</td>
<td>MyBP-C</td>
<td>Asp1064fsX38</td>
<td>SD</td>
<td>NA</td>
<td>Xin et al26</td>
</tr>
<tr>
<td>cTnT</td>
<td>Phe110Ile</td>
<td>cTnT</td>
<td>Phe110Ile</td>
<td>No</td>
<td>21 (49)</td>
<td>Lin et al27</td>
</tr>
<tr>
<td>cTnT</td>
<td>Ser179Phe</td>
<td>cTnT</td>
<td>Ser179Phe</td>
<td>SD</td>
<td>25 (17)</td>
<td>Ho et al28</td>
</tr>
</tbody>
</table>

IVS indicates interventricular septum; SD, sudden death event; HTx, heart transplant; and NA, not available.
double heterozygous mutation in the MyBP-C gene have also been reported and seem to also correlate with more severe disease. In one report, a baby with severe hypertrophy who died suddenly at age 5 weeks was found to carry a compound heterozygous mutation in the MyBP-C gene. The normal mother had a single base insertion causing a reading frame shift, leading to a premature translation stop, whereas each mutation in the single heterozygous state causes only mild HCM. This supports the collective data that, like homozygous HCM patients, double heterozygous patients display more clinically severe disease in HCM compared with single heterozygous patients.

**Compound Heterozygous FHC Mutations**

Most reports of multiple mutation carriers in HCM to date have involved compound heterozygous mutations (ie, when 2 mutations are identified in the same gene) (Table 4). This was first reported in a proband with a nonsense mutation and missense mutation on different βMHC alleles, but only those family members heterozygous for the missense mutation were clinically affected. The nonsense mutation did not show a dominant phenotype. One possibility is that the mutation might not be translated and incorporated into the sarcomere. In a second report, a boy aged 6 years, who had marked left ventricular hypertrophy and suffered a cardiac arrest, had a de novo Arg719Trp mutation on the paternally inherited βMHC allele and a Met349Thr mutation on the maternally inherited βMHC allele. The Arg719Trp mutation has been widely reported as causing severe HCM, with a mortality rate of 50% by age 38 years. The Met349Thr mutation was present in 5 asymptomatic family members and has been reported as a polymorphic variant in the normal population, making its pathogenicity in this family unclear.

**Multiple Mutations in Other Genetic Cardiovascular Disorders**

Although the number of reports are limited to date, it seems that up to 5% of families with HCM have multiple mutations, which include homozygous, double heterozygous, and compound heterozygous carriers. Collectively, these multiple mutation HCM patients have clinically more severe disease, including earlier age of disease onset, more severe left ventricular hypertrophy, and more frequent and rapid progression to the most significant complications of HCM (ie,
heart failure and sudden death. This suggests identification of 2 disease-causing mutations in an affected individual may be an additional marker in terms of risk stratification and may identify a subgroup of patients who may require more aggressive treatment and initiation of prevention strategies.

Although multiple mutations have been most frequently reported in HCM, it seems the notion of 2 disease-causing gene mutations causing more severe clinical disease is not restricted to only HCM. Recent reports in a number of other genetic cardiovascular disorders have implicated a role of multiple mutations in disease severity. Two examples are briefly mentioned here.

**Multiple Mutations in FH**

FH is a common inherited disease caused by dominant single mutations in any one of several genes that affect receptor-mediated uptake of low-density lipoproteins, such as the low-density lipoproteins receptor, apolipoprotein B100, and neural apoptosis-regulated convertase 1. The prevalence of FH in the general population is estimated to be between 0.2%
and 0.5%. FH heterozygotes have elevated low-density lipoproteins particle concentrations in plasma from birth up to 3 times greater than unaffected individuals and increasing with age.40 From approximately the third decade of life these patients also develop xanthomatous lesions and have a greater risk of myocardial infarction.

The prevalence of patients with multiple FH disease-causing mutations is estimated at 1 in 1 million people in the general population.39 This condition is commonly referred to as homozygous FH, although the term compound heterozygote is more fitting as many of these individuals inherit a different disease-causing mutation from each parent. The inheritance of multiple FH-causing mutations results in a more severe phenotype and is consistent with the findings in patients with multiple mutations causing HCM. Specifically, patients with multiple FH-causing mutations develop disease earlier in life, with plasma low-density lipoprotein particle concentrations being elevated 6- to 8-fold during intrauterine life.40 Furthermore, multiple mutation FH patients develop the characteristic xanthomatous lesions in the first years of life. It is rare for these FH patients to live past the age of 30 years because of the high risk of myocardial infarction due to extensive coronary atherosclerosis.

**Familial LQTS**

Familial LQTS is an autosomal dominantly inherited disease with a reported prevalence of $1 in 5000$, although it is likely that this is an underestimate.41 Mutations in at least 10 genes affecting $Na^+$, $K^+$, and $Ca^{2+}$ ion currents in cardiomyocytes are known to cause familial LQTS. Symptoms can include syncope and sudden death due to ventricular fibrillation, however, $\approx 30\%$ of LQTS single mutants are asymptomatic gene mutation carriers with a normal QT interval.41,42

Recent case reports of both compound and double heterozygous LQTS patients show they have more severe disease, with an earlier age of disease onset and a more prolonged QT interval in double-mutant patients compared with single-mutant family members.43 A recent study by Tester et al.42 screened 5 cardiac channel genes in 541 unrelated LQTS patients and found the prevalence of double-mutations to be 5% and that these double-mutant patients were significantly younger at diagnosis.

**Molecular Pathogenesis of Multiple Mutation Phenotypes**

The presence of multiple mutations in a number of different genetic cardiovascular disorders, and the association of multiple mutations with a more severe clinical phenotype, suggests the presence of 2 mutations has an additive effect in terms of molecular pathogenesis. As more genotype-phenotype correlation studies are performed in these multiple mutation cohorts, an important step is to understand the underlying mechanisms of how mutations interact to cause more profound disease and how these interactions could be modulated to improve clinical outcomes. To this end, studies in animal models are beginning to shed light on the role of multiple mutations in cardiovascular disease.

In HCM, a number of murine models have been developed over the last decade that support the clinical observation that multiple mutations lead to more severe HCM, associated with rapid progression to heart failure and premature death. Until recently, these models have predominantly involved homozygous mutations involving key cardiac sarcomere proteins. Two separate models of homozygote HCM mutations have been reported. In the first model, in which the $\alpha MHC$
Arg403Gln mutation is introduced by homologous recombination, heterozygous mice develop mild HCM by age 30 weeks with a normal lifespan.44–46 However, when bred to homozygosity, these mice develop a severe neonatal dilated cardiomyopathy, with death within 8 days of birth.47 In the second model, in which a truncated form of MyBP-C is introduced, heterozygote mice develop mild HCM by age 30 to 50 weeks with a normal lifespan.48 However, when bred to homozygosity, these mice develop a dilated cardiomyopathy early in life, although there is cardiac compensation and lifespan is normal.48

Most recently, the first mouse model of double heterozygous HCM was reported in Circulation.49 In brief, 2 previously reported mouse models of HCM (ie, the cTnI Gly203Ser and αMHC Arg403Gln models) were crossed to develop a double-mutant genotype (designated TnI-203/MHC-403). By age 14 days, the TnI-203/MHC-403 double-mutant mice develop a severe phenotype characterized by cardiac hypertrophy followed by 4-chamber cardiac dilatation, heart failure, severe myocyte disarray and interstitial fibrosis, and inducible ventricular tachycardia (Figure 3B). By age 21 days, mortality is 100%. In beginning to understand the underlying molecular mechanisms in this severe model of double-mutant HCM, preliminary results suggest a significant downregulation of key Ca^{2+} regulation genes (ryanodine receptor, L-type Ca^{2+} channel, SERCA2a, and phospholamban) as well as a cardiac-specific upregulation of the phosphorylated (active) form of the latent transcription factor signal and activator of transcription-3 (STAT-3) with onset of disease.49

This double-mutant model replicates the subgroup of human HCM patients (Table 5) who have severe heart failure requiring aggressive treatment, including transplantation. Further investigation of key animal models of HCM caused by multiple mutations will be important to investigate the mechanisms involved in HCM and to help elucidate possible mechanistic links between HCM and dilated cardiomyopathy. Development of cellular and animal models of other genetic cardiovascular disorders caused by multiple mutations will be an important step in our understanding of the role of gene-gene interactions and how this translates to the clinical phenotype.

**Impact of Multiple Mutations on Clinical Practice**

The identification of multiple mutations in individual patients with a number of different inherited cardiovascular disorders has a major impact on genetic diagnosis, highlights the importance of genetic counseling in families, and raises the possibility that such multiple mutation carriers may require more aggressive and targeted treatment.

The role of genetic testing and counseling is clearly an important component of the evaluation, diagnosis, and management of individuals and families with genetic cardiovascular diseases. The identification of multiple mutations among individual patients directly impacts on the genetic testing strategy.6 For example, whole panels of genes rather than single-gene testing should be carried out in new families presenting with disease. The implications to a family of not identifying a second gene mutation could be devastating. For example, an individual with a family history of HCM who receives a negative predictive gene test is released from clinical screening and believe their children are no longer at risk of developing HCM.

In addition, a key aspect of the genetic counseling process is the explanation of the inheritance of the disease and risk to children. The presence of multiple mutations in an autosomal disorder such as HCM or familial LQTS, alters the chances of inheritance in subsequent testing of offspring because there are now 3 possibilities of what the offspring may inherit (ie, no mutation, 1 mutation, or 2 mutations). This is illustrated in the family described in Figure 3A. The proband in this family is a double heterozygote carrying a gene mutation on 2 different genes inherited independently. Therefore, the risk that her child will receive at least 1 disease-causing gene mutation becomes 75%, with a 25% chance that the child will inherit both gene mutations. These are important considerations for a family and highlight the importance of accurate genetic testing of the proband. Of equal importance is ensuring cardiologists dealing with families with an inherited cardiovascular disorder are equipped with the skills to offer appropriate genetic counseling, perhaps as part of a multidisciplinary cardiac genetic service.5-50

The impact on treatment and prevention strategies in those who carry multiple mutations currently remains unclear. Although there is a clear association of multiple mutations with a more severe clinical phenotype in HCM, familial LQTS, and FH, further genotype-based prospective studies are required to fully evaluate the use of multiple mutation information for risk stratification purposes. For example, the presence of multiple mutations in some cardiovascular diseases which can lead to sudden death may become part of a risk stratification algorithm, leading to the earlier initiation of prevention therapies such as β-blockers or insertion of an implantable cardioverter-defibrillator.

One of the key issues that needs to be resolved in the field of multiple mutations in human disease is determining whether the DNA variants identified are truly pathogenic, rather than common silent polymorphisms of no functional significance. It is possible that in some cases, including ones already reported in the literature, both DNA variants are not causative. Therefore, rigorous methods and approaches will be required to determine the pathogenicity of each DNA variant identified in these patients. Traditional factors which may support the notion that a DNA variant is causative need to be investigated, including coinheritance with disease,

### Table 5. Comparison of Phenotype in Humans and Mice

<table>
<thead>
<tr>
<th>Clinical Feature</th>
<th>HCM Patients With Multiple Mutations</th>
<th>Double-Mutant Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival</td>
<td>Decreased (frequently &lt;40 y)</td>
<td>Decreased (21 d)</td>
</tr>
<tr>
<td>Early onset</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Left ventricular hypertrophy</td>
<td>Yes</td>
<td>Minimal</td>
</tr>
<tr>
<td>Progression to dilation</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Ventricular tachycardia</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Increased sudden death</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>
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conservation of the altered amino acid across species, and absence of the variant in normal populations. Furthermore, assays and methods to demonstrate functional significance, including both cellular and animal models, will all assist in determining the pathogenicity of the DNA variants identified and their role in clinical disease.

Conclusions

The occurrence of multiple gene mutations in patients with inherited cardiovascular diseases has changed the long-standing paradigm that a single mutation in a single gene is the cause of disease. Patients with multiple mutations have been found to develop more severe clinical disease, including earlier onset of disease, and more rapid development of disease complications including progressive heart failure and premature death. This suggests an additive effect of these multiple gene mutations on cardiovascular pathology and function and highlights the importance of basic molecular, cellular, and animal model studies to elucidate the key pathogenic pathways. The presence of multiple mutations has a direct impact on genetic diagnosis strategies as well as genetic counseling approaches whereby the chances of inher- itance in offspring are altered depending on the type of multiple mutations. Although not established currently, the presence of multiple gene mutations may play a role as a marker of disease severity and therefore identify patients who would most benefit from earlier treatment and prevention strategies.

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References


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