Familial Hypertrophic Cardiomyopathy
From Mutations to Functional Defects

Gisèle Bonne, Lucie Carrier, Pascale Richard, Bernard Hainque, Ketty Schwartz

Abstract—Hypertrophic cardiomyopathy is characterized by left and/or right ventricular hypertrophy, which is usually asymmetric and involves the interventricular septum. Typical morphological changes include myocyte hypertrophy and disarray surrounding the areas of increased loose connective tissue. Arrhythmias and premature sudden deaths are common. Hypertrophic cardiomyopathy is familial in the majority of cases and is transmitted as an autosomal-dominant trait. The results of molecular genetics studies have shown that familial hypertrophic cardiomyopathy is a disease of the sarcomere involving mutations in 7 different genes encoding proteins of the myofibrillar apparatus: β-myosin heavy chain, ventricular myosin essential light chain, ventricular myosin regulatory light chain, cardiac troponin T, cardiac troponin I, α-tropomyosin, and cardiac myosin binding protein C. In addition to this locus heterogeneity, there is a wide allelic heterogeneity, since numerous mutations have been found in all these genes. The recent development of animal models and of in vitro analyses have allowed a better understanding of the pathophysiological mechanisms associated with familial hypertrophic cardiomyopathy. One can thus tentatively draw the following cascade of events: The mutation leads to a poison polypeptide that would be incorporated into the sarcomere. This would alter the sarcomeric function that would result (1) in an altered cardiac function and then (2) in the alteration of the sarcomeric and myocyte structure. Some mutations induce functional impairment and support the pathogenesis hypothesis of a “hypocontractile” state followed by compensatory hypertrophy. Other mutations induce cardiac hyperfunction and determine a “hypercontractile” state that would directly induce cardiac hypertrophy. The development of other animal models and of other mechanistic studies linking the genetic mutation to functional defects are now key issues in understanding how alterations in the basic contractile unit of the cardiomyocyte alter the phenotype and the function of the heart. (Circ Res. 1998;83:580-593.)

Key Words: familial hypertrophic cardiomyopathy • genetic mutation • mouse model • sarcomeric protein • alteration in cardiac contractility

Observations of myocardial diseases that can reasonably be interpreted as hypertrophic cardiomyopathy were made in the middle of the last century at the Hôpital La Salpêtrière in Paris by A. Vulpian, who called what he saw at the macroscopic level a “rétrécissement de l’orifice ventriculo-aortique” or “sub-aortic stricture.”1 However, it was only in the late 1950s that the unique clinical features of hypertrophic cardiomyopathy were systematically described. It is characterized by left and/or right ventricular hypertrophy, which is usually asymmetric and which can affect different regions of the ventricle. The interventricular septum is most commonly affected, with or without involvement of either the anterior wall or the posterior wall in continuity. A particular form of regional involvement affects the apex but spares the upper portion of the septum (apical hypertrophy).2 Typically, the left ventricular volume is normal or reduced. Systolic gradients are common. Typical morphological changes include myocyte hypertrophy and disarray surrounding the areas of increased loose connective tissue. Arrhythmias and premature sudden deaths are common.3-4 Although hypertrophic cardiomyopathy has been regarded largely as a relatively uncommon cardiac disease, the prevalence of echocardiographically defined hypertrophic cardiomyopathy in a large cohort of apparently healthy young adults selected from a community-based general population was reported 3 years ago to be as high as 0.2%.3,4 Familial disease with autosomal-dominant inheritance predominates, and the first large pedigree of familial hypertrophic cardiomyopathy (FHC) was reported in 1960.6 None of the previous hypotheses of the pathophysiological mechanisms would have predicted that defects in sarcomeric genes could be a possible molecular basis for the disease. The results of molecular genetic studies have nevertheless shown that all mutations found so far concern sarcomeric proteins: 3 myofilament proteins, the β-myosin heavy chain (β-MyHC), the ventricular myosin essential light chain 1 (MLC-1s/v), and the ventricular...
myosin regulatory light chain 2 (MLC-2s/v); 3 thin-filament proteins, cardiac troponin T (cTnT), cardiac troponin I (cTnI), and α-tropomyosin (α-TM); and, finally, 1 myosin binding protein, the cardiac myosin binding protein C (cMyBP-C) (Table). These genes certainly do not represent the whole spectrum of FHC disease genes since an additional locus was reported, and one might reasonably hypothesize that disease genes yet to be identified include additional components of the sarcomere. One of the next challenges is to decipher the mechanisms through which the disease results from sarcomeric gene defects. The focus of this article is to review the mechanisms through which the disease results from sarcomeric gene defects. The focus of this article is to review the current knowledge of FHC disease genes and proteins and (2) the in vitro and in vivo functional consequences of these mutations.

**Organization and Mutations of FHC Disease Genes**

All the disease genes code proteins that are part of the sarcomere, which is a complex structure with an exact stoichiometry and multiple sites of protein–protein interactions. Myosin is the molecular motor that transduces energy from the hydrolysis of ATP into directed movement and that, by doing so, drives sarcomere shortening and muscle contraction. Cardiac myosin is a conventional class II myosin that consists of 2 heavy chains (MyHCs) and 2 pairs of light chains (MLCs), referred to as essential (or alkali) light chains (MLC-1) and regulatory (or phosphorylatable) light chains (MLC-2), respectively (reviews in References 8 and 9). The myosin molecule is highly asymmetric, consisting of 2 globular heads joined to a long rodlike tail. The light chains are arranged in tandem in the head-tail junction. Their function is not fully understood. Neither MLC type is required for the ATPase activity of the myosin head, but they probably modulate it in presence of actin and contribute to the rigidity of the neck, which is hypothesized to function as a lever arm for generating an effective power stroke. Mutations were found in the heavy chain and in the 2 types of ventricular light chains. The troponin complex and tropomysin constitute the calcium-sensitive switch that regulates the contraction of cardiac muscle fibers. Mutations were found in the α-TM and in 2 of the subunits of the troponin complex: cTnI, the inhibitory subunit, and cTnT, the tropomysin binding subunit. As for cMyBP-C, its function is uncertain, but for a decade, evidence has existed to indicate both structural and regulatory roles. Partial extraction of cMyBP-C from rat skinned cardiac myocytes and rabbit skeletal muscle fibers alters calcium-sensitive tension, and it was shown that phosphorylation of cMyBP-C alters myosin crossbridges in native thick filaments, suggesting that cMyBP-C can modify force production in activated cardiac muscles. Myosin and MyBP-C are part of the thick filaments of the sarcomere, with MyBP-C being located at the level of the transverse stripes, 43 nm apart, seen by electron microscopy in the sarcomere A-band (Figure 1). Troponins and tropomyosin are located in the thin filaments. Each of these proteins is encoded by multigene families, which exhibit tissue-specific, developmental, and physiologically regulated patterns of expression.

The organization of the human FHC disease genes and the sequences of at least all exon-intron boundaries are known for MYH7, MYL3, MYL2, TNNT2, TNNT3, and MYBP3, which encode β-MyHC, MLC-1s/v, MLC-2s/v, cTnT, cTnI, and cMyBP-C, respectively. For all these genes, mutational analysis in FHC can therefore be performed on genomic DNA. As for TPM1, which encodes α-TM, the genomic organization is known only in the rat. The genomic and protein organizations presented below were computed from the GENBANK, GDB, EMBL, PIR, and SWISSPROT databanks. The nomenclature of the genes and proteins varies from one databank to the other. For the sake of simplicity and clarity, we have used the nomenclature of GDB for the genes and the nomenclature of Schiaffino and Reggiani and of Gulick et al for the proteins. The amino acid numbering includes the NH2-terminal meth-
Human MYH7

β-MyHC is the major isoform of the human ventricle and of slow-twitch skeletal fibers (review in Reference 9). It is also expressed in the human atria. The other cardiac isoform is α-MyHC, which is predominantly expressed in the human atria and which, in mouse and rat hearts, predominates in both atria and ventricles. The α-MyHC isoform is encoded by the MYH6 gene. The MYH6 and MYH7 genes are organized in tandem in a cluster on chromosome 14q11.2-q13, with MYH7 being located 4 kb upstream from MYH6. As shown on Figure 2, MYH7 is composed of 40 exons, 38 of which are coding, and encompasses ~23 kb of DNA. It encodes a protein of 1935 amino acids. The globular amino-terminal part (subfragment 1 [S1]) corresponds to the motor domain that contains the ATP binding site and the actin binding site. Recent studies conducted on a skeletal/smooth chimeric myosin showed that the ATPase cycle rate (thus, the ATP hydrolysis rate or phosphate release rate) is solely determined by the globular head domain. The neck region of MyHC, which consists of a long 8.5-nm α-helix, is associated with the light chains (review in Reference 8). The COOH-terminal rod, which has a characteristic α-helical coiled-coil structure with 7-residue and 28-residue repeats, is responsible for the assembly of myosin into thick filaments. MYH7 contains 2 polymorphic dinucleotide repeats, one in the promoter region and one in the 24th intron, that we have named MYOI and MYOII, respectively, and that allow easy linkage analysis in FHC families.

At least 50 mutations were found in unrelated families with FHC (Figure 2), and 3 hot spots for mutations were identified, codons 403, 719, and 741. All but 3 of these mutations are missense mutations located either in the head or in the head-rod junction of the molecule. The 3 exceptions are two 3-bp deletions that do not disrupt the reading frame, one of codon 46 and the other of codon 930, and a 2.4-kb deletion in the 3′ region. In the kindred with the latter mutation, only the proband had developed clinically diagnosed hypertrophic cardiomyopathy at a very late onset (age, 59 years). Finally, a termination codon at position 54 was found by chance in the mother (38 years old) and the grandmother (70 years old) of an affected child (16 years old) who had also inherited from his affected father a missense mutation in exon 22. The allele corresponding to the nonsense mutation should encode a short variant of the β-MyHC comprising only the first 53 residues of the molecule, yet it does not present the characteristics of a disease-causing mutation, since the 2 women were clinically unaffected.

To examine the structural consequences of the mutations in the MYH7 gene, 29 mutations have been precisely positioned on the 3-dimensional structure of chicken skeletal myosin S1, which is expected to be very similar to that of the human β-MyHC S1. Twenty-four mutations do not appear to occur randomly in the structure but, rather, to cluster to 4 discrete localizations: the actin binding interface, around the nucleotide binding site, adjacent to the region that connects 2 reactive cysteine residues, and, finally, in proximity to the interface of the heavy chain with the essential light chain. The remaining 5 are in the myosin rod.

Human MYL3

The ventricular myosin essential (or alkali) light chain, MLC-1s/v, is expressed both in the ventricular myocardium and in the slow-twitch muscles. It belongs to the superfamily of EF-hand proteins, which includes calmodulin and troponin C (review in Reference 55). Thus, it folds into a dumbbell-like structure shape, with 2 helix-loop-helix structural motifs in each half of the molecule, which indicate the existence of 4 putative calcium binding sites (review in...
Reference 56). Indeed, in scallop myosin (but not in human ventricular myosin), calcium binding by the myosin essential light chain switches the motor on.57 In chicken skeletal muscle, removal of the myosin essential light chain slows the velocity at which skeletal muscle myosin moves actin in a motility assay58 and reduces isometric force by 50%. 59

Synthetic peptides corresponding to the NH2 terminus of human MLC-1s/v were recently shown to increase contractility of intact and chemically skinned human heart fibers 60 and to induce supramaximal stimulation of rat myofibrillar ATPase activity under specific stoichiometric conditions. 61 These data strongly suggest that MLC-1s/v potently produces an inotropic effect through a cooperative mechanism that may involve activation of the entire thin filament.

**MYL3** is located on chromosome 3p21.2-p21.3. It is composed of 7 exons, 6 of which encode a polypeptide of 195 amino acids62 (Figure 3). Six functional domains were putatively characterized, an actin binding site, a proline-rich region, and 4 helix-loop-helix regions. Because the 3-dimensional structure of chicken S1 demonstrated that this MyHC region bearing a cluster of mutations represents an interface with MLC-1s/v,48 Poetter et al63 hypothesized that mutations in this MLC might result in similar abnormalities in a subset of families with FHC. By screening DNA from representatives of 383 unrelated families with FHC, these authors found 2 missense mutations in exon 4, one in all affected members of a family and the other in an unrelated individual (Figure 3). Half of these patients (7 of 14) exhibited a rare phenotype involving mid left ventricular chamber thickening.

**Human MYL2**

The ventricular myosin regulatory light chain, MLC-2s/v, is expressed both in the ventricular myocardium and in the slow-twitch muscles.64 Like the essential MLC, it belongs to the superfamily of EF-hand proteins, and its removal significantly decreases the velocity of actin movement on skeletal myosin.58 However, at variance with MLC-1, removal of skeletal MLC-2 has little effect on isometric force.59 Phosphorylation of MLC-2 by MLC kinase is essential for actin-myosin interaction in smooth muscles, but it has only a modulator role in striated muscles.65 Recent studies conducted in the mouse highlighted the role of MLC-2s/v.13,66 The partial transgene-driven replacement of MLC-2s/v with the skeletal isoform reduces both left ventricular contractility and relaxation, although the unloaded shortening velocity of isolated ventricular cardiomyocytes is not significantly different.13 The disruption of the MLC-2s/v gene results in sarcomeric disassembly and in an embryonic form of dilated cardiomyopathy, indicating that there is a selective requirement for MLC-2s/v in the normal development of ventricular cardiac myocyte structure and function.66

**MYL2** is located on chromosome 12q23-q24.3, 67 and we have recently refined its localization on the genetic map, in an interval of 6 centimorgans containing 6 informative microsatellites.68 MYL2 encompasses 12 kb of genomic DNA; it is composed of 7 exons, all of which are coding ones (Figure 3). The encoded polypeptide is composed of 166 amino acids. Five active sites have been characterized, a phosphorylation site on serine 15 in exon 2 and 4 EF-hand domains, one of which has retained the ability to bind a metal ion and which is most likely occupied by Mg2+ in vivo.69 Five mutations were reported (Figure 3)63,68: 2 of them (E22K and A13T) are associated with the same rare phenotype seen with mutations in **MYL3** (see above) involving mid left ventricular chamber thickening,63 whereas mutations F18L and R58Q are associated with familial and classical forms of hypertrophic cardiomyopathy.68

**Human TNNT2**

In human cardiac muscle, multiple isoforms of cTnT have been described that are expressed in the fetal, adult, and diseased heart and that result from alternative splicing of the single gene **TNNT2**.70-72 The precise physiological relevance of these isoforms is currently poorly understood. **TNNT2** was mapped by somatic cell hybrid analysis and by fluorescent in
situ hybridization to chromosome 1q32.\textsuperscript{71,73} The structural organization and the complete nucleotide sequence have been determined in the rat by Jin et al.\textsuperscript{74} and the first mutations reported in FHC were numbered according to this rat structure.\textsuperscript{75} We have partially established the organization of the human gene, and this allows us now to precisely identify the position of the mutations within exons, including those alternatively spliced during development, and also to use an amino acid numbering that reflects the full coding potential of human $\text{TNNT2}$.\textsuperscript{76}

$\text{TNNT2}$ is composed of 17 exons spread over 17 kb and gives rise to multiple isoforms by the use of both alternative exons and alternative acceptor sites (Figure 4). The cardiac isoform shares the overall structure and function of other troponin isoforms. It is an asymmetric molecule of $\approx 37$ kDa whose elongated NH$_2$-terminal part extends for a considerable length along tropomyosin and spans the head-to-tail overlap of tropomyosin (review in Reference 77). It contains several putative functional domains: a phosphorylation site in the NH$_2$-terminal region, an $\alpha$-tropomyosin binding region between exons 9 and 12, and in the COOH-terminal part a region that binds in a calcium-dependent manner $\alpha$-tropomyosin, troponin C, and troponin I and that contains 3 phosphorylation sites.\textsuperscript{77} The phosphorylation of cTnT results in an inhibition of the maximum ATPase rate of reconstituted thin-filament preparations showing that cTnT plays a role in the regulation of crossbridge kinetics (review in Reference 77). Eleven mutations were found in unrelated FHC families;\textsuperscript{75,76,78–80} 3 of which are located in a hot spot (codon 102) (Figure 4).\textsuperscript{75,76,79} Ten mutations are missense ones located between exons 9 and 17.\textsuperscript{75,76,78–80} One mutation is a 3-bp deletion located in exon 12 that does not disrupt the coding frame, and the last is located in the intron 16 splice donor site and is predicted to produce a truncated protein in which the C-terminal binding sites are disrupted.\textsuperscript{75}

**Human $\text{TNNI3}$**

The cTnI isoform is expressed only in cardiac muscles.\textsuperscript{81} $\text{TNNI3}$, located on chromosome 19p13.2-q13.2,\textsuperscript{82} comprises 8 exons contained within 6.2 kb of genomic DNA (Figure 4). It encodes a polypeptide of 210 amino acids that shows a high degree of homology with the fast-skeletal and slow-skeletal isoforms in the COOH-terminal region, whereas the NH$_2$-terminal region is more divergent.\textsuperscript{83,84} Cooperative binding of cTnI to actin-tropomyosin is a unique property of the cardiac variant (review in Reference 85). cTnI contains several functional domains: (1) the NH$_2$-terminal extension that contains 2 sites at serine residues 23 and 24, the phosphorylation of which alters calcium sensitivity and eliminates cooperative binding to actin, (2) the near NH$_2$-terminal domain that binds to the COOH terminus of cardiac troponin C and that contains 2 sites at serine residues 42 and 44, the phosphorylation of which reduces the maximum ATPase rate, (3) the inhibitory region that binds to actin and to cardiac troponin C and that causes relaxation through inhibition of the actomyosin interaction, and (4) the COOH-terminal domain, which is essential for the calcium sensitivity of the myofilaments.\textsuperscript{85,86} Six mutations were recently identified (Figure 4).\textsuperscript{84} Five are missense mutations located in exons 7 and 10 (Figure 4).
and 8, and one is a K183Δ mutation that does not disrupt the coding frame.

**Human \(\text{TPM1}\)**

\(\text{TPM1}\) encodes several isoforms generated by alternative splicing (review in Reference 87). The cardiac isoform is expressed both in the ventricular myocardium and in fast-twitch skeletal muscles. It shares the overall structure of other tropomyosins that are rodlike proteins that possess a simple dimeric α-coiled-coil structure in parallel orientation along their entire length. The coiled-coil structure is based on a repeated pattern of 7 amino acids, with hydrophobic residues at the first and fourth positions. These dimers are arranged in a head-to-tail fashion, lie in the major groove of actin filaments, and span 7 actin monomers.

The chromosomal localization of \(\text{TPM1}\) is known in humans (15q22) and not in rats, but its complete organization has been determined in rats and not in humans (review in Reference 87). \(\text{TPM1}\) is composed of 14 exons, with exons 1a, 2b, 3, 4, 5, 6b, 7, 8, 9a, and 9b being expressed in the cardiac tissue (Figure 4). The encoded polypeptide is composed of 284 amino acids and contains 2 putative tropoinin T binding domains that attach α-TM to the troponin complex, one near C190, where troponin C and troponin I occur, and one along the COOH-terminal stretch of the molecule (Figure 4). Four missense mutations were found in unrelated FHC families. Two of them, A63V and K70T, are located in exon 2b within the consensus pattern of sequence repeats of α-TM and could alter tropomyosin binding to actin. Mutations D175N and E180G are both located within constitutive exon 5, in a region near the C190 and near the calcium-dependent tropoinin T binding domain.

**Human \(\text{MYBPC3}\)**

Human \(\text{MYBPC3}\) was localized by fluorescent in situ hybridization on chromosome 11p11.2, and its precise position was determined by radiation hybrid mapping between loci \(D11S4133\) and \(D11S1326\). We have recently determined its organization and sequence (Figure 5). It comprises >21 kb and contains 35 exons, out of which 34 are coding. Two exons are unusually small in size, 3 bp each. The full-length 4.5-kb cDNA encodes a 1173-residue polypeptide that shares the overall modular architecture of the intracellular immunoglobulin superfamily and contains 8 IgI modules and 3 fibronecin-3 domains. Three distinct regions are specific to the cardiac isoform: the NH2-terminal domain C0 IgI containing 101 residues, the MyBP-C motif (a 105-residue loop inserted in the C5 IgI domain), and a 28-residue loop present in the tissue and act in a dominant fashion, probably as poison polypeptides; ie, they are incorporated in the sarcomere and change the function of the wild-type protein and/or the assembly of the sarcomeric filaments. Resolution of mutant from wild-type protein has typically not been possible because of the subtle nature of most mutations and the limited access to myocardial specimens. The incorporation of mutant protein in vivo has been nevertheless demonstrated for 2 mutations, the R403Q \(\text{MYH7}\) mutation that could be distinguished in extracts from skeletal muscle because it induces the loss of an arginine specific endoproteinase digest site and the D175N \(\text{TPM1}\) mutation because it presents a specific electrophoretic band in skeletal muscle from affected patients. This poison polypeptide hypothesis is also supported by a variety of results obtained in vitro (see below) and by findings in nematodes in which missense mutations produce stable polypeptides that are incorporated into myofibrils and disrupt the sarcomere assembly.

The situation is more complex when mutations leading to truncated proteins are involved. The mutations could induce “null alleles” potentially leading to haploinsufficiency: the production of insufficient quantities of a normal sarcomeric protein would produce an imbalance in the stoichiometry of the thick- or the thin-filament components that would be sufficient to alter the sarcomeric structure and function. In this case, the null alleles would exhibit a dominant phenotype. This is what occurs in *Drosophila*, where heterozygotes...
for actin or myosin null alleles have complex myofibrillar defects, whereas double heterozygotes for both actin and myosin null alleles where stoichiometry is maintained have nearly normal myofibrils. \(^{109}\) This is also what occurs in heterozygous mice for \(\alpha\)-MyHC null alleles that have severe impairment of both contractility and relaxation. \(^{110}\) However, none of the available data in FHC are consistent with a mechanism of haploinsufficiency. A nonsense mutation has been found in the \(\text{MYH7}\) gene that is predicted to encode a short variant of \(\beta\)-MyHC protein (53 residues) in 2 healthy individuals (38 and 70 years old). \(^{47}\) This indicates that the single normal human cardiac \(\text{MYH7}\) allele is sufficient to compensate for the heterozygous defect of the null allele.

Two other recent studies addressed the null allele hypothesis, one by expressing truncated human cTnT in quail myotubes\(^{111}\) and the other by characterizing the transcripts and proteins present in an endomyocardial biopsy of a patient with a cMyBP-C splice donor site mutation. \(^{101}\) None of the results were consistent with a mechanism of haploinsufficiency. The cTnT mutation appeared to function as a dominant-negative allele, maybe because the truncated cTnT still contained one tropomyosin binding site. Whether the truncated cTnT protein is expressed and stable in myocardial tissue from affected patients with FHC remains to be determined. As for the cMyBP-C, the authors suggest that the mutation could produce a misfolded RNA template that may interfere with the formation of ordered sarcomeres already on the mRNA level. \(^{101}\) More studies are necessary to understand the molecular mechanisms by which mutations predicted to lead to truncated proteins cause FHC.

How Mutations Alter Sarcomere Function and Lead to Hypertrophic Cardiomyopathy

The fact that mutations in different components of the sarcomere appear to produce the same phenotype suggests that the mutations share functional consequences that lead to
a common mode of pathogenesis of FHC. One of the major problems in our understanding of FHC has been the difficulty in obtaining cardiac specimens from affected patients. This has generated the development of animal models and of in vitro analyses to study the pathophysiology of mutations.

**Mouse Models of FHC**

Two mouse models of FHC have been published as full studies. They were produced by the introduction of the R403Q MYH7 mutation into the mouse MYH6 gene (the adult mouse ventricle contains only α-MyHC and no β-MyHC). In one model, the R403Q mutation was introduced by homologous recombination; thus, heterozygous mice carry one normal gene and one mutated gene. Both models represent heterologous systems, because the human β-MyHC gene mutation was introduced into the mouse α-MyHC. α-MyHC (high ATPase activity) is adapted to the mouse heart, and β-MyHC (low ATPase activity) is adapted to the human heart. The functional consequences of the mutations observed in the mouse myocytes may not thus represent exactly what occurs in human myocytes. In spite of this, analysis of these genetically different animal models allows us to make hypotheses regarding the pathophysiological mechanisms of the mutated α-MyHC molecules and to extrapolate them to the β-MyHC molecules.

In α-MyHC80/81 mice, the R403Q MYH7 gene mutation was introduced in the mouse MYH6 gene by a “hit-and-run” homologous recombination technique. Homozygous mutant mice died 7 days after birth. Heterozygous mice survived and presented cardiac dysfunction at 5 weeks of age, characterized by a modification of the left ventricular pressure and a prolonged relaxation time. Histological alterations similar to those seen in FHC patients appeared at 15 weeks of age, including myocyte disarray, myocyte hypertrophy, and fibrosis, suggesting that the altered mechanical properties may lead to the histological changes. At 15 weeks of age, no ventricular hypertrophy was observed, whereas atrial enlargement was present, which is at variance with what occurs in FHC patients. Further investigations of electrocardiographical and electrophysiological parameters demonstrated a modified QRS axis, heterogeneous ventricular conduction, and arrhythmia. All these modifications evolved with time to a more pronounced phenotype in males than in females, but without ventricular hypertrophy. Since humans with FHC may experience life-threatening events with exercise, Geisterfer-Lowrance et al. evaluated the impact of physical activity on the phenotype by a swimming test in a subset of five α-MyHC80/81 mice. One mouse died suddenly while swimming; its heart was grossly enlarged with a thrombus in the dilated atrium, mild right ventricular hypertrophy, and marked asymmetric left ventricular hypertrophy.

In the transgenic mice, the transgene carrying both the R403Q MYH7 mutation and a deletion in the actin binding site of α-MyHC was transcribed at 26% to 50% of the endogenous α-MyHC mRNA level. Although the mutant protein represented only 1% to 12% of the heart’s myosin, the mice exhibited the cardiac histopathology seen in FHC patients, namely, myocyte disarray and hypertrophy, sarcomeric disorganization, and, finally, left and right ventricular hypertrophy. In addition, 2 molecular markers of compensatory hypertrophy, ANF and α-skeletal actin genes, were upregulated. Evolution of the phenotype with age showed differences between males and females, with an evolution toward a severe left ventricular hypertrophy for the females, whereas males developed ventricular dilation. In contrast to the α-MyHC80/81 model, no atrial enlargement was found.

These 2 mouse models give us several clues. First, they confirm the hypothesis of the dominant-negative effect of the R403Q MYH7 gene mutation. Indeed, for the 2 models, the mutant protein is synthesized, stable, and present, although in variable amounts. The second message is that modifications of MYH6 expression result in alterations of the normal cardiac function and structure. This is in agreement with what was found in the in vitro analyses of the R403Q MYH7 mutation, which showed an impairment of the function (see below). One can thus tentatively draw the cascade of events that would lead from the R403Q myosin mutation to the phenotype. Incorporation of a poison protein would alter sarcomeric function, which would result first in an altered cardiac function and then in the alteration of the sarcomeric and myocyte structures. Obviously, the alteration of the sarcomeric structure would contribute to the emphasis of the cardiac dysfunction, since sarcomeres presented both abnormal structure and function. Finally, both abnormal structure and cardiac dysfunction lead to the compensatory response of the heart that develops hypertrophy, the last manifestation observed in the 2 models. The third message is the incomplete penetrance of the phenotypes as well as the sex-related evolution of the disease. This suggests the implication of other “actors,” such as modifier genes and/or environmental factors, that could modulate the phenotypic expression of the mutated genes.

**In Vitro Analyses of the Consequences of FHC Mutations**

**Structural Aspects**

The expression of β-MyHC and cTNT mutant proteins in primary cultures of ventricular cardiomyocytes or quail myotubes does not appear to have major structural consequences. Indeed the R403Q and the R249Q MYH7 mutations were cloned and expressed in primary cultures of neonatal rat cardiomyocytes. Mutants were readily incorporated into cardiac cells and did not disrupt myofilaments even after prolonged exposure. In another study, however, the R403Q MYH7 mutation was expressed in adult feline cardiac myocytes and resulted in a disruption of the sarcomere structure in 50% of the myocytes 5 days after being exposed to a high dose of adenoviral vector; whether the β-MyHC mutant protein is incorporated into sarcomeres had not been determined. It is unclear why the same mutant β-MyHC, R403Q, produced different in vitro structural consequences. Becker et al. suggest that myofibrils in adult feline myocytes may be more sensitive to disruption by the R403Q mutant β-MyHC or that the neonatal rat cardiomyocytes
system may not accurately mimic the mechanical load found in vivo, which may be necessary to produce disarray. As for cTnT, the expression in adult feline cardiomyocytes of the R102Q TNNT2 mutation located in one of the α-TM binding domains showed an integration of the cTnT mutant in the sarcomere without significant disruption of its structure 48 hours after transfection with adenoviral vector.117 Almost similar conclusions were drawn by Watkins et al,111 who showed an integration of the COOH-terminal truncated cTnT mutant in the primary culture of quail myotubes and only focal disruption of sarcomeric organization in 14% to 21% of the cells. That study111 is at variance with the study involving the analogous Drosophila mutation, which induced a rapid degradation of flight muscle troponin T leading to myofibrillar misassembly.120

**Functional Aspects**

In contrast to structural analyses, several in vitro functional studies have shown that FHC mutants alter sarcomere function, either by decreasing the translocating filament activity and/or force leading to a reduction of power production by cardiac cells31,105,111,115,144 or, on the contrary, by increasing in vitro motility rates of filament sliding and/or force.63,106,121–123

Nine MYH7 mutations, T124I, Y162C, R249Q, G256E, R403Q, R453C, V606M, R870H, and L908V, resulted in a decrease of the sliding of the actin filament in the in vitro motility assay.116,144 Cuda et al116 suggest that several mechanisms could explain this slower motility: (1) the mutant might affect the crossbridge cycling rate by interfering with the kinetic rate constant that limits sliding velocity; (2) the crossbridge kinetics might be unaltered, but the distance in which the actin filament is moved per crossbridge stroke might be shortened; and (3) the mutated head of myosin might be noncycling yet still able to bind to actin and act as an internal load on the movement of the normal crossbridges present in the assay. Other studies performed on skinned slow skeletal muscle fibers from affected patients with FHC have shown that fibers containing the G741R or the R403Q MYH7 mutations exhibit decreased maximum shortening of velocity and decreased isometric force generation.115 The calcium-activated force of contraction was also found depressed in quail myotubes expressing a C-terminal truncated cTnT mutant protein.111 This latter study demonstrates that in contrast to what was found for the analogous Drosophila mutation,120 the truncated cTnT that causes FHC does not act as a null allele but probably acts as a dominant-negative allele, blocking full calcium activation of the thin filament. Another expressed TNNT2 mutation, R102Q, located in the α-TM binding domain, has been shown to result in an impairment of the contractile performance of adult feline cardiomyocytes (reduced fractional shortening and peak velocity of shortening).117 Finally, mutants of Dicystelium discoideum myosin II equivalent to 6 human FHC mutations, R403Q, F513C, G584R, G716R, R719Q, and R719W, were generated by site-directed mutagenesis.121 The mutant Dicystelium discoideum myosins showed impaired function determined by reduction of force, affinity to actin, and ATPase activity, and these data could be correlated to the prognosis of individuals affected by the corresponding FHC mutation. The authors have thus proposed that the force level of the mutant myosin molecule may be one of the key factors for pathogenesis responsible for the prognosis of human FHC. All these data suggest that some FHC mutations induce functional impairment rather than disruption of the sarcomeric structure and support the FHC pathogenesis hypothesis of a “hypocontractile” state, in which mutations induce functional cardiac impairment followed by compensatory hypertrophy.

Not all FHC mutations result in decreased in vitro motility filament translocation. Ventricular myosin from patients with either the R719Q MYH7 or the M149V MYL3 mutations displayed faster than normal in vitro actin sliding rates.63 The R719Q MYH7 mutation lies near the interface of the β-MyHC with the MLC-1s/v, suggesting that the topography of the mutation may be an important factor contributing to the variability of function alteration. For MYL2 gene mutations, preliminary data showed that ventricular myosin from patients with the E22K mutation gives actin translocation velocities that are indistinguishable from those of control myosin.63 This mutation is close to the MLC-2s/v phosphorylation site, and this result is at variance with what was found in Drosophila melanogaster, in which mutations of the phosphorylation sites of the skeletal muscle MLC-2s/v are associated with a large reduction of both the power output and force in isolated skinned fibers.125 The authors suggest that MLC-2s/v phosphorylation may be required for forming the myosin-actin interaction or that it may also affect the mobility or position of the myosin head to promote actomyosin interaction. A recombinant rat cTnT mutant, corresponding to the I89N TNNT2 mutation in humans, which is located in the 5′ end of the gene outside of the α-TM binding region, also resulted in a 50% faster thin-filament movement over a surface coated with heavy meromyosin in in vitro motility assays121; however, neither the binding of the troponin complex with the other thin-filament components nor the interactions between thick and thin filaments were altered.121 As for α-TM, its D175N mutant (affected near the calcium-dependent cTnT binding domain) increased the velocity of actin translocation in an in vitro motility assay.122 Other data obtained on skinned fibers or with recombinant mutant proteins did not show any impairment of the sarcomeric function. This is the case for the G256E MYH7 mutation, which had contractile properties that were indistinguishable from normal,123 as well as for the skewed skeletal fibers of 2 patients containing the D175N TPM1 mutation, which showed an increase of the calcium sensitivity of the skeletal muscle fibers.106 and for the recombinant D175N mutant protein, which showed a reduction of the actin affinity with altered conformation when bound to actin in the S1-induced on-state of the thin filament.121 So the mutant protein is expressed and almost certainly incorporated into muscle in vivo and does result in altered contractile function; this confirms a dominant-negative rather than a null allele action. Because the mutant α-TM was associated with an increased calcium sensitivity, this mutation might be associated more with an enhancement than a depression of cardiac contractile performance. Taken altogether, these data suggest that some
mutations might determine a “hypercontractile” state that would directly induce cardiac hypertrophy.

It should be pointed out that not only the hypercontractile hypothesis but also the more common hypocontractile hypothesis are still somewhat tentative and incomplete. Indeed, in most studies and for practical reasons, the in vitro motility assay has been used to determine the function of mutant proteins. Because velocity is determined in the absence of load, it is not clear what a decrease or increase in velocity in motility assays would necessarily mean in terms of force generation or power output under load, since the heart always works under load in vivo. Only 3 studies have reported force measurements, and even though these results involved human skinned muscle fibers, which to date correspond to the “most physiological” conditions, they should be considered with caution since the experiments were carried out at low temperature. Finally, these data further emphasize the need to analyze the contractility for other FHC mutants and the development of other animal models to better understand the pathogenesis of FHC.

Does the Genotypic Heterogeneity of FHC Account for its Phenotypic Heterogeneity?

Before genetic studies, it was well known that the pattern and extent of left ventricular hypertrophy vary greatly even in first-degree relatives and that a high incidence of sudden death occurs in selected families. Genetic studies have provided insights into the heterogeneity of FHC clinical features. However, the results must be seen as preliminary, because the available data relate to only a few hundred individuals, and it is obvious that although a given phenotype may be apparent in a small family, examining large or multiple families with the same mutation is required before drawing unambiguous conclusions. Nevertheless, several concepts begin to emerge, at least for mutations in the MYH7, TNNT2, and MYBPC3 genes. For MYH7, it is clear that prognosis for patients with different mutations varies considerably (for review see Reference 128). For example, the R403Q mutation appears to be associated with markedly reduced survival, whereas some other mutations, such as V606M, appear more benign. The disease caused by TNNT2 mutations is usually associated with a 20% incidence of nonpenetrance, a relatively mild and sometimes subclinical hypertrophy, but a high incidence of sudden death, which can occur even in the absence of significant clinical left ventricular hypertrophy. In one family with a TNNT2 mutation, however, penetrance is complete, echocardiographic data show a wide range of hypertrophy, and there has been no sudden cardiac death. Mutations in MYBPC3 seem to be characterized by specific clinical features with a mild phenotype in young subjects, a delayed age at the onset of symptoms, and a favorable prognosis before the age of 40. Genetic studies have also revealed the presence of clinically healthy individuals carrying the mutant allele, which is associated in first-degree relatives with a typical phenotype of the disease. In some cases, as many as a quarter of genetically affected individuals do not express the disease. Several mechanisms could account for the large variability of the phenotypic expression of the mutations: the degree of functional impairment caused in the sarcomere by the mutation (that may vary markedly with the position of the mutation in the molecule and the type of protein involved), the role of environmental differences and acquired traits (eg, differences in lifestyle, risk factors, and exercise), and the existence of modifier genes and/or polymorphisms that could modulate the phenotypic expression of the disease. The only significant results obtained so far concern the influence of the angiotensin I–converting enzyme insertion/deletion polymorphism. Association studies showed that the D allele is more common in patients with hypertrophic cardiomyopathy and in patients with a high incidence of sudden cardiac death than in a control population. We recently showed that the association between the D allele and hypertrophy is observed in the case of MYH7 R403 codon mutations but not with MYBPC3 mutation carriers, raising the concept of multiple genetic modifiers in FHC.

The genetic status begins to be used as the criterion of reference to reassess diagnostic criteria and penetrance. The diagnosis of FHC is usually based on ECG and echocardiography, and it is generally considered that echocardiography is a more accurate technique than ECG for the diagnosis in adults. Analysis of a large genotyped population recently showed that, in fact, ECG and echocardiography have similar diagnostic values for FHC in adults, with an excellent specificity and a lower sensitivity. As for penetrance, it was a much-debated issue. Before molecular genetic analyses, several studies have indicated either a full penetrance or an incomplete one. We have recently reassessed the penetrance of FHC in a large genotyped population and found that it is incomplete, age-related, and greater in males than in females. These latter data have very important implications for genetic counseling, especially for women under the age of 50. The transgenic mouse model of FHC also shows a gender difference, and it now provides a good genetic model for determining the direct role of sexual hormones on myocardium and for studying the role of putative modifier genes on sexual chromosomes.

Conclusions and Future Directions

Although we have only begun to dissect the molecular mechanisms leading to FHC, this review illustrates that mutations in sarcomeric genes are now recognized as a principal cause of this disease. The present focus on sarcomeric proteins should not, however, preclude the search for other genetic origins: the finding that both hypertrophic and dilated cardiomyopathies in hamsters are caused by mutations in the δ-sarcoglycan gene encoding a protein of the dystrophin-associated glycoprotein complex could provide significant new insights into the pathogenesis of hypertrophic cardiomyopathy in humans. Conversely, it was recently reported that mutations in another sarcomeric gene, cardiac actin, cause dilated cardiomyopathy in humans. These 2 sets of data raise the important issue of whether hypertrophic and dilated cardiomyopathies are inherently independent diseases or whether dilation is part of the FHC spectrum. The demand for molecular genetic testing will certainly increase as physicians and patients become better educated as to how these data can influence patient care and family-planning
decisions. Given the large genetic heterogeneity of the disease, new strategies of genetic testing need to be developed. Since the disease genes that cause FHC have been identified only recently, the continued characterization of patient mutations and their phenotypes is necessary to establish clinically meaningful correlations. Finally, the development of other animal models and of other mechanistic studies linking the genetic mutation to functional defects are now key issues in understanding how alterations in the basic contractile unit of the cardiomyocyte alter the phenotype and the function of the heart.

Note Added in Proof
Tardif et al have created transgenic mice expressing a cTnT allele with a carboxyl-terminal truncation (Tardiff JC, Factor SM, Tompkins TE, Hewett TE, Palmer BM, Moore RL, Schwartz S, Robbins J, Leinwand L. A truncated cardiac troponin T molecule in transgenic mice suggests multiple functional defects of the heart. J Clin Invest. 1991;101:2800–2811). The hearts of these animals are smaller than wild types because of a primary loss of myocytes and a decrease in myocardial cell size. In addition, these animals exhibit significant diastolic dysfunction. This novel mouse model of FHC provides a model system to investigate the relationship between geometric changes in the heart and the severity of the phenotype.

Acknowledgment
We are grateful to Dr Marc Fiszman for a careful reading of the manuscript.

References


Familial Hypertrophic Cardiomyopathy: From Mutations to Functional Defects
Gisèle Bonne, Lucie Carrier, Pascale Richard, Bernard Hainque and Ketty Schwartz

Circ Res. 1998;83:580-593
doi: 10.1161/01.RES.83.6.580

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circres.ahajournals.org/content/83/6/580

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published
in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the
Editorial Office. Once the online version of the published article for which permission is being requested is
located, click Request Permissions in the middle column of the Web page under Services. Further information
about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/