Molecular and phenotypic effects of heterozygous, homozygous, and compound heterozygote myosin heavy-chain mutations

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Submitted 30 June 2004; accepted in final form 28 October 2004


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mutant β-MHC associated with individuals having one or two mutant alleles. With two mutant alleles, expression of mutant myosin in which both heavy chains carry a mutation will exist, which may help define the impact of the mutation on myosin molecular function. In addition, the penetrance and severity of the disease may be gene-dose dependent, which would result in patients with a more severe clinical phenotype. Here we have identified several patients with point mutations, with one being novel, in both β-MHC alleles. These patients were uniformly of a severe FHC phenotype. Mutant myosin prepared from biopsy of the upper portion of the biceps muscle obtained from these patients and family members was characterized for the effect of the mutation on myosin’s ability to move actin in a motility assay.

**MATERIALS AND METHODS**

*Patients and genetic analysis.* Patients with FHC and their family members in pedigrees in which two mutant MYH7 alleles were previously identified were studied. Informed consent was obtained under protocols approved by the Institutional Review Boards of the National Heart, Lung, and Blood Institute (99-H-0065 and 98-H-0100) and the University of Vermont (IRB00000485).

Clinical studies included two-dimensional echocardiography, 12-lead electrocardiography, and in selected cases, cardiac magnetic resonance imaging. An individual was considered positive for the FHC phenotype if LV wall thickness was >13 mm by echocardiography in the absence of another cause for cardiac hypertrophy. Genetic analysis was performed on genomic DNA extracted from whole blood (PureGene; Gentra) as per the manufacturer’s instructions, and genotype was determined by direct sequencing and confirmed by restriction enzyme digestion when possible. Restriction enzyme cleavage was predicted by using MapDraw (SeqMan; DNASTar). Penetrance (%) was defined as (phenotype and genotype positive individuals/ genotype positive individuals) × 100.

*Biopsy and myosin preparation.* Skeletal muscle myosin was isolated from samples of human biceps tissue (~10 mg) obtained through a small incision over the upper portion of the biceps muscle in the nondominant arm under local anesthesia. The biceps muscle is made up of ~40–60% slow fibers (19) that express the cardiac β-MHC isoform. Therefore, the isolated myosin will be a mixture of both normal and mutant heavy chains as well as fast and slow skeletal myosin isoforms. This concern was mitigated by comparison of motility results for myosins isolated from the biceps versus cardiac and soleus tissues (see RESULTS). Myosin purification from small tissue samples for use in the in vitro motility assay has been previously described in detail (22, 23, 33). With the limited sample size, the quantity of purified myosin only allowed in vitro motility experiments to be performed.

*In vitro motility assay.* Standard methods were used for the in vitro motility assay with special care taken to remove all nonfunctional myosin (23, 35). This last step is critical so that the internal load contributed by nonfunctional myosin is eliminated. Actin prepared from chicken skeletal muscle was fluorescently labeled by incubation in tetramethylrhodamine isothiocyanate-phalloidin overnight (24, 35). Assays were carried out at 30°C using a 30-μl chamber to which the following solutions were added and removed (23) 1) 100 μg/ml myosin; 2) bovine serum albumin; 3) 1 μM unlabeled actin in actin buffer (in mM: 25 KCl, 25 imidazole, 1 EGTA, 4 MgCl₂, and 10 dithiothreitol, plus oxygen scavengers, pH 7.4); 4) actin buffer with 1 mM MgATP; 5) 6 × 30 μl washes with actin buffer; 6) 10 mM labeled actin; and 7) 1 mM MgATP in actin buffer with 0.375% methylcellulose. Step 3 and a comparable step in the initial myosin isolation procedure remove denatured, rigor-like, nonfunctional myosin that might act as a load to the free movement of actin filaments in the motility assay (13, 23). Actin movement was visualized, recorded, and digitally analyzed to determine actin filament velocity or velocity of actin translocation (Vactin) (35, 36).

**RESULTS**

Genetic testing and clinical correlates of the molecular defects. Two FHC pedigrees with two mutant MYH7 alleles were identified. The mutant alleles were Lys207Gln/Lys207Gln (K207Q/K207Q) in family A (Fig. 1) and Leu908Val/Asp906Gly (L908V/D906G) in family B (Fig. 2). The D906G is a novel mutant reported here for the first time. Mutants K207Q and L908V have been reported previously (5, 18).

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**Fig. 1.** Structure of the family with MYH7 mutation Lys207Gln/Lys207Gln (K207Q/K207Q). The proband is marked with an arrow. The numbering of individuals correspond to that in Table 1.

**Fig. 2.** Structure of the family with MYH7 mutations Asp906Gly (D906G) and Leu908Val (L908V). Only family members with a mutant allele are shown; genotype negative individuals are excluded for clarity and to promote anonymity.
In family A (Fig. 1), the proband was homozygous for K207Q, a nonconservative mutation in a loop spanning the ATP-binding pocket. This surface loop is highly variable across the different classes of myosins from various species and organisms and therefore the sequence variation may dictate myosin enzymatic and mechanical function (20). However, the lysine at residue 207 is conserved across all mammalian cardiac myosins, regardless of isoform (1).

The proband, who presented with palpitations, had been diagnosed with FHC at the age of 47 yr. Both parents are assumed to be heterozygous, and there is no historical evidence that they were related. His father died in his mid-40s with a history strongly suggestive of FHC, but his mother died at the age of 92 yr. The proband has survived to his 70s, but his FHC has been complicated by chronic atrial fibrillation (AF), multiple episodes of syncope, and therapeutic interventions with a defibrillator-cardioverter device. Serial echocardiograms demonstrate progressive LV dilatation and systolic impairment and reduction in septal thickness, severe left atrial (LA) enlargement, and the development of pulmonary hypertension. No other family member was homozygous and nine were heterozygous, including a sister who was subsequently diagnosed with FHC at the age of 80 yr. She has a history of frequent presyncope and syncope and a septal wall thickness of 38 mm with midventricular obstruction. There was no history of hypertension. Eight other family members heterozygous for K207Q had not developed LV hypertrophy at the ages of 46, 43, 40, 18, 15, 14, 13, and 9 yr (20% penetrance over the age of 18 yr). Five of these individuals have a resting sinus bradycardia, suggesting an alternative phenotypic expression (Table 1).

For the second family (Fig. 2), a four-generation pedigree was founded after two brothers from a family with FHC married two sisters from another family also with FHC. We have established that each family had a different MYH7 mutation affecting the rod region of β-MHC, a conservative substitution L908V, and D906G, a charge-changing substitution. Both the 906 and 908 residues are conserved across all mammalian cardiac isoforms (1). Two descendants have both L908V and D906G and several others have either L908V or D906G. Both L908V/D906G compound heterozygotes developed FHC, but the penetrance for L908V/wild type (WT) was 46% (21/46), and 25% (3/13) for D906G (WT/WT) moved actin with a value not significant). The two double heterozygotes both had prominent mid-LV cavity hypertrophy, moderate-to-severe LA enlargement, and paroxysmal AF. LV phenotype in penetrant obligate heterozygous genotypes.

**Table 1. Cardiac findings in individuals heterozygous for K207Q**

<table>
<thead>
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<th>Individual</th>
<th>Age, yr</th>
<th>Heart Rate, beats/min</th>
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<td>80</td>
<td>81</td>
<td>HCM</td>
</tr>
<tr>
<td>III-1</td>
<td>46</td>
<td>56</td>
<td>Normal</td>
</tr>
<tr>
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<td>45</td>
<td>Normal</td>
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<td>74</td>
<td>Normal</td>
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<tr>
<td>IV-1</td>
<td>18</td>
<td>91</td>
<td>Normal</td>
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<tr>
<td>IV-3</td>
<td>15</td>
<td>54</td>
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</tr>
<tr>
<td>IV-5</td>
<td>9</td>
<td>48</td>
<td>Normal</td>
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</tbody>
</table>

Fig. 3. Velocities of actin translocation ($V_{actin}$) produced by mutant (L908V) and wild-type β-myosin extracted from biceps, cardiac, and soleus muscle biopsies are compared. Biceps and cardiac muscle myosins produce similar $V_{actin}$, suggesting biceps is a suitable source for β-myosin heavy chain. The cardiac and soleus data have been published previously (12).

from mild LV hypertrophy to severe LV hypertrophy with LV outflow obstruction. Sudden death and defibrillator discharges were also reported for several individuals with proven or obligate heterozygous genotypes.

**In vitro motility assay.** The effects of the MYH7 mutations on myosin mechanical function were assessed by characterizing skeletal muscle myosin from biceps muscles. This approach was similar to that previously used by ourselves and other investigators (3, 23). The biceps express β-MHC as well as fast skeletal myosin isoforms. To control for any differences in actin filament velocities resulting from differential expression of the fast skeletal, normal, and mutant β-MHC rather than an effect due to the mutant myosin itself, we compared the results from our previous studies of the L908V mutation isolated from both heart and soleus muscle biopsies to data for the L908V myosin from the biceps (Fig. 3) (3, 23).

Normal myosin from control samples containing the β-MHC (WT/WT) moved actin with a $V_{actin}$ of $1.4 \pm 0.1 \mu m/s$, whereas myosin from biceps containing the L908V/WT mutant moved actin 21% faster ($P < 0.05$, Figs. 3 and 4). The increased $V_{actin}$ for L908V agrees qualitatively with our previous observation of faster $V_{actin}$ for the L908V when isolated from either heart (in which no skeletal myosin is expressed) or soleus tissue samples (Fig. 3) (3, 23). Thus any confounding effects due to the known mixture of fast and slow myosin isoforms that are normally expressed in the biceps (19) are minimal, allowing the biceps to serve as a valid tissue preparation for assessing mutant myosin mechanical performance.

Enhanced $V_{actin}$ was also observed for D906G/WT β-MHC in which $V_{actin}$ was $1.9 \pm 0.1 \mu m/s$ or 34% faster than control ($P < 0.005$, Fig. 4). In contrast, L908V/D906G β-MHC had $V_{actin}$ similar to controls ($1.5 \pm 0.1 \mu m/s$). Thus the effect on $V_{actin}$ of the individual mutations do not appear to be additive and, in fact, may even cancel each other. A similar result was observed for the K207Q/K207Q mutation with $V_{actin}$ of 1.5 μm/s being similar to normal values. However, because myo-
isolations were performed. With two isolations, the average
from which samples were obtained. For each patient sample either one or two

tions. For each of the samples obtained from these patients,
duplicate motility experiments were performed and the average
reported for an individual.

**DISCUSSION**

The homozygote and double-heterozygote patients in this
and other reports have a clinical course characterized by either
severe LV hypertrophy (21, 27) and/or progressive LV systolic
dysfunction, severe LA enlargement, and AF (18, 25, 26),
suggesting a gene-dose effect on the severity of phenotypic
expression. The phenotypic progression to LV dilatation and
systolic impairment is relatively uncommon, described in only
~10% of FHC patients (16, 30). Animal models exist that
demonstrate that the cardiac pathologies of the heterozygote
and homozygote “malignant” sarcomeric protein mutations
result in significantly different cardiomyopathies (7, 17). To
our knowledge, no nonpenetrant homozygote or double het-

erozygotes have been reported in humans or homozygous
transgenic animal models, consistent with a view that gene
dosage also affects penetrance (7, 17).

The considerable variation of hypertrophy and other mani-
festations of FHC in individuals heterozygous for the same
mutation is well described (14). Notably, in our pedigrees there
were some severe expressions of FHC despite the very low
penetrance, suggesting that uncharacterized modifying mech-

isms exert powerful influences. It is reasonable to expect that
the effects of these modifying factors are most prominent when
a mutant protein’s functional abnormalities are mildest. Ac-


![Fig. 4. Comparisons of the $V_{\text{actin}}$ for control (wild type) and mutant myosin preparations. *-test significance at $P < 0.05$; $n$, number of different patients from which samples were obtained. For each patient sample either one or two isolations were performed. With two isolations, the average $V_{\text{actin}}$ for the 2 experiments are reported for a given patient.](http://ajpheart.physiology.org/)

The D906G is reported here for the first time. We previously
found no amino acid changing sequence abnormalities after
screening many exons encoding the motor region of $\beta$-MHC in
100 normal controls (200 alleles) (18). This screening included
exon 23, encoding D906 and is consistent with the view that
the D906G is a mutation and not a polymorphism, and that
there may be no amino acid changing polymorphisms in
$\beta$-MHC’s entire motor region.

Assaying myosin’s molecular mechanics allows us to test
the hypothesis that the extent of alterations to mutant myosin
function may be related to one or more measurements of the
severity of the FHC cardiac phenotype, such as the degree of
penetrance, magnitude of hypertrophy, or incidence of sudden
death. If so, then measurable parameters of myosin molecular
motor function may contribute to the prognostic evaluations for
FHC patients. Therefore, we isolated myosin from the biceps
muscle of patients with the following mutations: L908V/WT,
D906G/WT, L908V/D906G, and K207Q/K207Q and assessed
their ability to propel actin filaments in the in vitro motility
assay. This assay serves as a molecular model system to
characterize the actomyosin interaction that relates to unloaded
shortening in muscle. Of note, the L908V/WT and the
D906G/WT mutations enhanced rather than compromised my-
osin’s unloaded velocity-generating capacities. The enhanced
mechanical performance of the L908V/WT confirms our pre-
vious studies (23) of this mutation from human cardiac and
soleus samples. This earlier study (23) was unique in that the


n: 2; K207Q/K207Q, $n = 1$), it may not be possible to draw any statistical con-
clusions. For each of the samples obtained from these patients,
duplicate motility experiments were performed and the average
$V_{\text{actin}}$ reported for an individual.

![Fig. 4. Comparisons of the $V_{\text{actin}}$ for control (wild type) and mutant myosin preparations. *-test significance at $P < 0.05$; $n$, number of different patients from which samples were obtained. For each patient sample either one or two isolations were performed. With two isolations, the average $V_{\text{actin}}$ for the 2 experiments are reported for a given patient.](http://ajpheart.physiology.org/)

The L908V and D906G are located in the S2-segment of the
myosin rod, far from the motor domain in which ATP hydro-
lysis and actin binding occur. This raises the important ques-
tion of how the mutations in the S2 segment can affect the
mechanical performance of the motor domain. The potential
for long distance communication between the catalytic site and
domains either proximal or distal to this site is possible through
structural elements within the myosin motor domain (for ex-
amples see review in Ref. 34). Another explanation is that
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erozygous for either mutation. However, \( V_{\text{actin}} \) was similar to normal controls (Fig. 4). A trivial explanation for the lack of an effect is due to the limited number of patients \((n=2)\), which prevents any statistically definitive statements from being made. This, too, was the case for the single patient in which both MYH7 alleles code for the K207Q mutation (Fig. 4), a mutation that exists in a surface loop that spans the entrance to the nucleotide-binding pocket. Therefore, the true effect of having a double mutation on myosin function cannot be determined at this time, although the severity of the clinical phenotype for these patients strongly suggests that a mutational effect does exist for these myosins. The paucity of data on double mutants is due to the apparent rarity of such patients, and the probability that the same double mutations are present in other FHC pedigrees must be exceedingly low.

In the present study, \( V_{\text{actin}} \) was the only measured mechanical index due to the limited tissue sample size. Studies of a limited number of mutant myosins in which careful isolation procedures were exercised (see above) have demonstrated an increased \( V_{\text{actin}} \), suggesting that enhanced \( V_{\text{actin}} \) may contribute to the pathological abnormality leading to FHC (13, 23, 33, 37). However, the enhanced \( V_{\text{actin}} \) may not be universal for all myosin mutations associated with FHC, given that numerous other mutations have yet to be characterized. In fact, given the limited data set reported here for the compound mutations, enhanced function could not be determined and raises concern that \( V_{\text{actin}} \) may not be the mechanical index of choice. The normal functioning of the heart, though, is critically dependent on its ability to generate power in which power is defined as the product of force and velocity. Therefore, changes in \( V_{\text{actin}} \) alone may not represent the fundamental molecular abnormality that ultimately results in the FHC phenotype but rather alterations to power output. Given that muscle force and velocity are interdependent mechanical parameters, it is only possible to measure a muscle’s peak power output once the relationship between muscle velocity and load (i.e., force) has been determined. Power producing capacities for mutant myosins have yet to be determined in any previous studies, and it may be possible that a mutant myosin exhibits increased \( V_{\text{actin}} \), yet generates either reduced or greater power than normal myosin. Our future goal is to define the force-velocity relationship for mutant myosins at the molecular level in the laser trap. Such experiments may provide greater insight to the mutations’ effects on myosin function and better define how different mutations, e.g., mutations in \( \beta\)-MHC’s actin binding region may result in functional abnormalities distinct from those in other structural domains of the myosin molecule. Finally, a more precise description of myosin power production may uncover how the primary insult in myosin function leads to the development of the FHC phenotype, which is more severe in patients with double \( \beta\)-MHC mutations.

GRANTS

This work was funded by National Heart, Lung, and Blood Institute Grant P01-HL-59408 (to N. R. Alpert and D. M. Warshaw).

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