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# Mutations and sequence variation in the human myosin heavy chain IIa gene (*MYH2*)

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We recently described a new autosomal dominant myopathy (OMIM #605637) associated with a missense mutation in the myosin heavy chain (MyHC) IIa gene (*MYH2*). In this study, we performed mutation analysis of *MYH2* in eight Swedish patients with familial myopathy of unknown cause. In two of the eight index cases, we identified novel heterozygous missense mutations in *MYH2*, one in each case: V970I and L1061V. The mutations were located in subfragment 2 of the MyHC and they changed highly conserved residues. Most family members carrying the mutations had signs and symptoms consisting mainly of mild muscle weakness and myalgia. In addition, we analyzed the extent and distribution of nucleotide variation in *MYH2* in 50 blood donors, who served as controls, by the complete sequencing of all 38 exons comprising the coding region. We identified only six polymorphic sites, five of which were synonymous polymorphisms. One variant, which occurred at an allele frequency of 0.01, was identical to the L1061V that was also found in one of the families with myopathy. The results of the analysis of normal variation indicate that there is strong selective pressure against mutations in *MYH2*. On the basis of these results, we suggest that MyHC genes should be regarded as candidate genes in cases of hereditary myopathies of unknown etiology.

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## Introduction

Myosin is the main component of skeletal muscle thick filaments accounting for 15–25% of the total body protein.<sup>1</sup> It is a molecular motor that converts chemical energy released from ATP hydrolysis into mechanical force. Class II or conventional two-headed myosin is a hexamer consisting of two identical heavy chains and two pairs of non-identical myosin light chains. Myosin heavy chain (MyHC) is a highly asymmetric protein with a long rod

domain and a globular head. ATPase and actin-binding sites reside in the globular amino-terminal motor domain or subfragment 1 (S1), which is responsible for the force transduction properties of myosin. The larger C-terminal portion of the rod, termed light meromyosin (LMM), lies along the thick filament axis and mediates filament assembly. Subfragment 2 (S2), the N-terminal region of the myosin rod, which is predicted to have an alpha-helical coiled-coil structure, links S1 to the filament backbone. The LMM provides sites for the binding of myosin-associated proteins such as myosin-binding protein C (MyBP-C), M-protein, myomesin and titin.<sup>2–4</sup>

Three major MyHC isoforms are expressed in adult human limb muscle: MyHC I, also named slow/beta MyHC

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(encoded from *MYH7*), is expressed in slow, oxidative, type 1 muscle fibers; MyHC IIa (encoded from *MYH2*) is expressed in fast, intermediate, type 2A muscle fibers; and MyHC IIx (encoded from *MYH1*) is expressed in fast, glycolytic, type 2B muscle fibers.<sup>5</sup> *MYH1* and *MYH2* are located on chromosome 17 in a cluster together with *MYH3* (MyHC-embryonic), *MYH8* (MyHC-perinatal), *MYH4* (MyHC IIb) and *MYH13* (MyHC-extraocular).<sup>6,7</sup> Slow/beta MyHC is also the major isoform in cardiac ventricles of adult, large mammals,<sup>7</sup> including humans, and is located in chromosome 14, together with *MYH6* (alpha MyHC).<sup>7,8</sup>

There is a greater degree of conservation between the homologous isoforms of MyHC of different species than between any different isoforms within a given species. This observation suggests that selective pressure has acted to maintain functionally distinct MyHC isoform sequences among the MyHC genes.<sup>9</sup>

We recently described the first mutation in *MYH2*.<sup>10</sup> This was a heterozygous E706K mutation located in the SH1 helix in the core of the *MYH2* head and was associated with an autosomal dominant myopathy.<sup>11,12</sup> So far, no mutations have been reported in *MYH1*.

Here, we describe two novel heterozygous missense mutations located in the S2 region of the MyHC IIa in two patients with familial myopathies of unknown etiology. In order to improve the possibility to interpret the importance of *MYH2* mutations, we determined the extent and nature of normal *MYH2* sequence variation in a human population by sequencing the entire coding region of 50 individuals (100 alleles).

## Material and methods

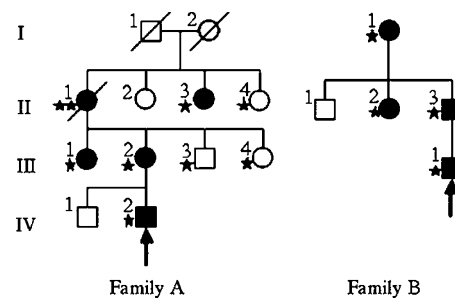
### Patients and controls

We screened for *MYH2* mutations in eight unrelated patients with familial myopathy of early onset and unknown etiology. In two of the families, we found two heterozygous missense mutations, which apparently segregated with muscle disease. The pedigrees of the two families with mutations are illustrated in Figure 1. The signs and symptoms from the neuromuscular system and the results of muscle biopsies are given in Table 1. The presence of the identified mutations was also analyzed in the DNA of 100 controls.

To analyze the sequence variation in *MYH2*, the DNA from peripheral blood leukocytes from 50 of these controls was investigated. These were blood donors of Caucasian origin, aged 18–60 year, and resident in Göteborg, Sweden.

### Genotype analysis

Genomic DNA was extracted from frozen skeletal muscle or peripheral blood using a DNA Extraction Kit (Qiagen, Hilden, Germany). The entire coding sequence of *MYH2* was analyzed in the patients and the 50 controls by



**Figure 1** Pedigrees of families A and B with the mutations. Only clinically investigated family members are included. Arrows indicate index cases. Squares: males; circles: females. Filled symbols correspond to the individuals with signs and symptoms according to Table 1. Presence of mutation is indicated by asterisks.

sequencing polymerase chain reaction (PCR) products amplified from exons 1 to 38 of genomic DNA. PCR analysis was performed in a master mixture (ReddyMix PCR Master Mix; Abgene, Epsom, UK) after the addition of 20 pmol of each primer and 50 ng DNA. The PCR amplifications were performed for 35 cycles at 94°C for 30 s, 57°C for 30 s and 72°C for 30 s. We used intronic primers according to Martinsson *et al*,<sup>10</sup> apart from using a different forward and reverse primer (18 + 19F: 5'-AGTTGT-TAAAAGCTATATGT-3' and 18 + 19R: 5'-TGGAATATGT-GAATAAACAT-3') for exons 18–19 and a different reverse primer (28R: 5'-ATGTCTTTCTGATTCAATAT-3') for exon 28. Nucleotide sequence determination was performed by cycle sequencing using a BigDye Terminator DNA sequencing kit on a 377 DNA sequencer (Applied Biosystems, Foster City, CA, USA).

### Restriction fragment length polymorphism (RFLP) analysis

A mismatch primer was designed to confirm the 2908G>A mutation in family A, using the removal of an *Sty1* restriction enzyme site. To confirm the 3181C>T mutation in family B by RFLP analysis, a mismatch reverse primer was used to introduce an *Hpa1* restriction enzyme site in the mutated allele. The mismatch primers, exon 21R mismatch: 5'-TGGCATGTTTCTCCTTCGGAA-3' and exon 23R mismatch: 5'-AACTTCAAGTCAAGTCACCGTTAA-3', were used with the opposite strand primers from the original intronic primer pairs.

### Web addresses and GenBank accession numbers

Autosomal dominant myopathy: OMIM #605637.

Blast analyses were performed using the NCBI BLAST web site:

(<http://www.ncbi.nlm.nih.gov/BLAST/>).

*MYH2* GenBank accession number: AF111784.

*MYH2* sequence of the German Genome Project with GenBank accession number: BX510904

(<http://mips.gsf.de/proj/cDNA/>).

**Table 1** Clinical and muscle morphological features in members of families A and B

Case	Onset of symptoms	Age at latest examination	Clinical history	Clinical features	Muscle biopsy findings	Presence of mutation
A II:1	30–40 yr	66 yr	Myalgia and muscle weakness	Mild shoulder and pelvic girdle weakness. Normal muscle tendon reflexes	Type 2 fiber atrophy	— <sup>a</sup>
A II:2	—	62 yr	No muscle symptoms	Normal	Not examined	— <sup>b</sup>
A II:3	30–40 yr	55 yr	Myalgia and muscle weakness	Mild weakness of axial, shoulder and pelvic girdle muscles. Normal muscle tendon reflexes	Not examined	— <sup>c</sup>
A II:4	30–40 yr	52 yr	No muscle symptoms	Normal	Not examined	— <sup>c</sup>
A III:1	20–30 yr	50 yr	Myalgia and generalized muscle weakness, proximal > distal	Mild weakness of shoulder and pelvic girdle muscles and hands. Normal muscle tendon reflexes	Not examined	— <sup>c</sup>
A III:2	20–30 yr	48 yr	Myalgia and muscle weakness in the arms, legs and back	Mild weakness and atrophy in the pelvic girdle and upper leg muscles. Increased cervicothoracic kyphosis. Weak Achilles tendon reflexes	Type 2 fiber atrophy	— <sup>c</sup>
A III:3	—	44 yr	Normal	Normal	Not examined	— <sup>c</sup>
A III:4	—	34 yr	Normal	Normal	Not examined	— <sup>c</sup>
A IV:1	—	27 yr	Normal	Normal	Essentially normal	— <sup>b</sup>
A IV:2	Childhood	23 yr	Myalgia in neck and back, and muscle weakness in the upper arms and back	Weakness and atrophy in the shoulder girdle muscles and scoliosis. Weak Achilles tendon reflexes	Essentially normal	— <sup>c</sup>
B I:1	40–50 yr	85 yr	Myalgia and back pain	Muscle weakness in shoulder girdle, upper arms and neck. Absent reflexes in the patellar and Achilles tendons	Not examined	— <sup>c</sup>
B II:1	—	61 yr	No muscle symptoms	Normal	Not examined	— <sup>b</sup>
B II:2	40–50 yr	59 yr	Myalgia and joint pain. Suspected Sjögren's syndrome	Mild muscle weakness in shoulder girdle and hands. Normal muscle tendon reflexes	Not examined	— <sup>c</sup>
B II:3	Adulthood	48 yr	Back pain	Mild kyphoscoliosis. Weak muscle tendon reflexes	Frequent lobulated muscle fibers (type 1)	— <sup>c</sup>
B III:1	Birth	7 yr	Weak fetal movements, bilateral club feet	Weakness and atrophy predominately in axial and shoulder girdle muscles. Weak muscle tendon reflexes	Type 1 fiber predominance, frequent central nuclei and mini-core changes	— <sup>c</sup>

<sup>a</sup>Homozygous.<sup>b</sup>No mutation.<sup>c</sup>Heterozygous.

## Results

### Mutation analysis in the myopathy patients

We performed mutation analysis of *MYH2* in eight unrelated patients with familial myopathy. The entire coding sequence of the gene was investigated in the index cases of each family. Two of these index cases revealed missense mutations in *MYH2* that apparently segregated with muscle disease in the family in question. The results are summarized in Table 1. In the index case of family A, as well as in his mother and all the mother's siblings, we identified a heterozygous missense mutation, 2908G>A, changing the nonpolar valine at position 970 to the nonpolar isoleucine. The patient's grandmother was homozygous for the mutation. The mutation was not identified in 200 control chromosomes. In the index case of family B, as well as several family members, we identified a heterozygous missense mutation, 3181C>T, changing the nonpolar leucine at position 1061 to the nonpolar valine. The mutation was also identified in two of 200 control chromosomes. We also identified a heterozygous missense mutation, 5780G>A, in the father of an index case in one of the other six families. However, this mutation was not present in the index case.

### Sequence variation analysis

Direct sequencing of all the 38 exons of *MYH2* in 50 individuals revealed six polymorphic sites (Table 2). Five of them were sequence variations without altering the amino acid. Four of them were located in the globular head region at sites 183, 189, 324 and 399, whereas one was located in the rod region at site 5034 of MyHC IIa. The allele frequency of the minor allele varied from 0.01 to 0.47. The sixth polymorphic site was located at position 3181. The major allele, which also corresponded to the GenBank sequence, had a frequency of 0.98. The minor allele, which was found in heterozygous form in two individuals, changed the nonpolar leucine at position 1061 to the nonpolar valine. This missense mutation was identical to that found in myopathy family B. In addition, we found

three nucleotide variants differing from the GenBank sequence at sites 9, 69 and 3390. They did not alter the encoded amino acid. The allele frequency was 1.0 for these variants and they were also identical to a recently published human *MYH2* sequence, clone DKFZp451A123 from the German Genome Project with GenBank accession number: BX510904 (<http://mips.gsf.de/proj/cDNA/>)

## Discussion

In spite of the fact that myosin is a major component of skeletal muscle, there are few reports of myopathies associated with mutations in MyHC genes. Mutations in the MyHC I gene (*MYH7*) are a frequent cause of familial hypertrophic cardiomyopathy. Although MyHC I is also expressed in skeletal muscle, the phenotypic expression of these mutations appears to be associated with mild skeletal myopathy. This has been described as being a central core-like myopathy from a morphological point of view.<sup>13</sup> Only one *MYH7* mutation has so far been associated with predominant skeletal myopathy with no or minor cardiomyopathy. This was a congenital myopathy with pathologic subsarcolemma accumulation of slow/beta myosin in type 1 muscle fibers.<sup>14</sup> The observation that *MYH7* mutations are mainly expressed as cardiomyopathies may be explained by the fact that only one major type of MyHC is expressed in cardiac ventricles, whereas there are three different MyHC isoforms in skeletal muscle. The effects of defective MyHC I may therefore be less pronounced in skeletal muscle compared with cardiac muscle.

MyHC molecules are considered to be very conserved in evolutionary term,<sup>9</sup> and almost all the more than 70 missense point mutations so far described in *MYH7* are pathogenic in heterozygous form and associated with cardiomyopathy (Human Gene Mutation Database: <http://archive.uwcm.ac.uk/uwcm/mg/search/120215.html>). Furthermore, the determination of the extent and distribution of naturally occurring polymorphisms in the *MYH7* coding sequence has shown that there is

**Table 2** Results of sequencing the entire *MYH2* coding sequence in 50 blood donors

Exon	Nucleotide	Amino acid	GenBank sequence (G)	Variant (V)	Genotypes (number of individuals)			Frequency of variant alleles (%) F(V)
					GG	GV	VV	
1	9	3	TCT (S)	TCA (S)	0	0	50	1.0
1	69	23	GAA (E)	GAG (E)	0	0	50	1.0
1	183	61	ACG (T)	ACA (T)	49	1	0	0.01
1	189	63	AAG (K)	AAA (K)	49	0	1	0.02
2	324	108	GAA (E)	GAG (E)	23	16	11	0.38
3	399	133	CCT (P)	CCA (P)	21	11	18	0.47
23	3181	1061	CTT (L)	GTT (V)	98	2	0	0.01 <sup>a</sup>
25	3390	1130	GCG (A)	GCA (A)	0	0	50	1.0
33	5034	1678	GCC (A)	GCT (A)	44	5	1	0.07

Polymorphic sites and variants compared with the GenBank sequences (accession number: AF111784) are indicated

<sup>a</sup>Based on 100 individuals.

very low sequence variation compared with most other human autosomal genes.<sup>15</sup> These findings have been interpreted as being a consequence of strong selective pressure against mutations in *MYH7*.<sup>15</sup> The results of our analysis of sequence variability in *MYH2* indicate that the frequency of polymorphic sites may be even lower than in *MYH7*.

Recently, we described the first mutation in *MYH2* (encoding MyHC IIa), a missense mutation associated with familial skeletal myopathy.<sup>10,12</sup> We hypothesized that many other pathogenic mutations in *MYH2* and also in *MYH1* (encoding MyHC IIx) may, in fact, segregate in humans and cause skeletal myopathy of varying severity. However, since only one family with myopathy associated with mutation in a fast MyHC isoform has been described, the cases in which myosin mutations should be suspected are not known. In this study, we investigated eight unrelated patients with familial myopathy of unknown cause to explore the hypothesis that these myopathies are, in fact, associated in some instances with *MYH2* mutations. We found two novel missense mutations in *MYH2*, which segregated with muscle disease in the two affected families.

In family A, a missense 2908G>A mutation was identified. It changed the nonpolar valine to the nonpolar isoleucine at position 970, which is located in the S2 region of MyHC IIa. This is an evolutionarily conserved amino acid among species with different skeletal muscle fiber types. In addition, the val970 is located immediately distal to the 126 conserved residues of S2, which has been demonstrated to be the binding site for the regulatory domain of MyBP-C in the slow/beta cardiac MyHC.<sup>16</sup> The regulatory function of MyBP-C is mediated by interaction with S2 and mutations in MyHC S2 may act by altering the interactions with MyBP-C.<sup>16</sup> In addition to the index case, the mutation was identified in the mother and all her siblings. The mother and one of her sisters have symptoms from the neuromuscular system, expressed as muscle weakness and myalgia. Two of the siblings carrying the mutation were healthy according to their history. The grandmother of the index case was not alive, but she had had a history of severe muscle complaints and had previously been subjected to muscle biopsy. She was homozygous for the mutation, which was not present in 200 control chromosomes. Her parents both originated from a small village in the central/western part of Sweden, indicating the possibility of consanguinity. Although the mutation appears to segregate with a familial myopathy, there are two arguments against a causal relationship between the 2908G>A mutation and the muscle disease in this family. First, two of the four siblings carrying the mutation had no symptoms of muscle disease. Second, case AII:1, who was homozygous for the mutation, appeared not to be more severely affected than the index case (AIV:2).

In family B, a missense 3181C>T mutation was identified. It changed the nonpolar leucine to the nonpolar valine at position 1061, which is located in the S2 region of MyHC IIa. This is an evolutionarily conserved residue among species with different skeletal muscle fiber types. All the family members who had experienced symptoms from the neuromuscular system, such as myalgia and muscle weakness and were available for genetic analysis, were heterozygous for the mutation. The mutation was also identified in two of 100 controls, with an allele frequency of 0.01, which is an argument against the pathogenicity of the 3181C>T mutation. These controls consisted of a group of blood donors at Sahlgrenska University Hospital in Göteborg, Sweden. Family B originates from and is living in Göteborg. Since the individual controls cannot be identified, the presence of signs or symptoms of muscle disease in the control population has not been excluded.

We have not been able to prove the pathogenicity of any of the two missense mutations. There are arguments in favor and against their pathogenicity. Our analysis of the sequence variability of *MYH2* indicates that the variability may be even lower in *MYH2* than in *MYH7*. In *MYH7*, the low variability has been interpreted as indicating strong selective pressure against mutations, which is also in accordance with the finding that MyHC genes are highly conserved during evolution.<sup>15,17</sup> In addition, almost all the more than 70 missense mutations that have been identified so far in *MYH7* are pathogenic and associated with cardiomyopathy. With a few exceptions, there is an apparent segregation of the mutations with signs and symptoms of muscle disorder in families A and B. These exceptions may be due to variable expression, which is also seen in *MYH7* mutations associated with cardiomyopathy.<sup>13</sup> There are several explanations for the variable expression of *MYH2* mutations. There are three major isoforms of MyHC in skeletal muscle, expressed in three different muscle fiber types. The fact that skeletal muscle expresses different and evolutionarily highly conserved MyHCs indicates that they are all functionally important. However, the unaffected muscle fibers may partially compensate for functional defects in one of the fiber types depending on the proportion of the defective myosin and the demands made of muscle function in an individual.

Our patients displayed only minor morphological changes in muscle biopsy specimens. However, defective molecular motor function, which is the major role of MyHC, does not necessarily result in morphological alterations. Pathogenic mutations in *MYH7*, as revealed by an abnormal *in vitro* motility assay of mutant beta-myosin obtained from skeletal muscle, do not always result in morphological alterations in muscle.<sup>13,18</sup> As a result, functional studies in cases of suspected pathogenic mutations in *MYH2* will be important to establish the pathogenicity of the mutations.

In conclusion, we have identified two novel *MYH2* missense mutations, which are possibly associated with muscle disorders. The clinical expression is variable and mild and may possibly depend on environmental and other factors. Future studies of familial myopathies of unknown cause, combined with studies of the functional consequences of identified mutations, will be necessary to establish the importance of MyHC mutations for muscle disabilities.

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