ARTICLE

Recessive myosin myopathy with external ophthalmoplegia associated with *MYH2* mutations

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Myosin myopathies comprise a group of inherited diseases caused by mutations in myosin heavy chain (MyHC) genes. Homozygous or compound heterozygous truncating *MYH2* mutations have been demonstrated to cause recessive myopathy with ophthalmoplegia, mild-to-moderate muscle weakness and complete lack of type 2A muscle fibers. In this study, we describe for the first time the clinical and morphological characteristics of recessive myosin IIa myopathy associated with *MYH2* missense mutations. Seven patients of five different families with a myopathy characterized by ophthalmoplegia and mild-to-moderate muscle weakness were investigated. Muscle biopsy was performed to study morphological changes and MyHC isoform expression. Five of the patients were homozygous for *MYH2* missense mutations, one patient was compound heterozygous for a missense and a nonsense mutation and one patient was homozygous for a frame-shift *MYH2* mutation. Muscle biopsy demonstrated small or absent type 2A muscle fibers and reduced or absent expression of the corresponding MyHC IIa transcript and protein. We conclude that mild muscle weakness and ophthalmoplegia in combination with muscle biopsy demonstrating small or absent type 2A muscle fibers are the hallmark of recessive myopathy associated with *MYH2* mutations.

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INTRODUCTION

Myosin heavy chain (MyHC) is a major structural component of the striated muscle contractile apparatus and is essential for body movement and heart contractility. There are several striated muscle MyHC isoforms encoded by different genes.¹ In adult human limb skeletal muscle, there are three major MyHC isoforms: MyHC I, also called slow/ β -cardiac MyHC, is the gene product of *MYH7* and is expressed in slow, type 1 muscle fibers as well as in the cardiac ventricles; MyHC IIa (*MYH2*) is expressed in fast, type 2A muscle fibers and MyHC IIx (*MYH1*) is expressed in fast, type 2B muscle fibers.² The three different muscle fiber types differ in their contractile and physiological properties. In addition, embryonic MyHC (*MYH3*) and fetal MyHC (*MYH8*) are myosin isoforms expressed during muscle development and in regenerating muscle fibers.^{3,4} Fetal MyHC expressing fibers may be observed also in other pathologies.

Mutations in MyHC isoforms have been associated with different muscle disease.^{5–7} These include autosomal dominant myopathy with congenital joint contractures, ophthalmoplegia and rimmed vacuoles (OMIM #605637), which was first reported as inclusion body

myopathy (IBM3), caused by a single point mutation in the fast IIa MyHC gene (*MYH2*);^{8–10} recessive myopathy with ophthalmoplegia because of truncating *MYH2* mutations;¹¹ Laing early-onset distal myopathy (OMIM #160500)^{12,13} and myosin storage myopathy (OMIM #608358),¹⁴ caused by different mutations in the slow/ β -cardiac MyHC gene (*MYH7*); Trismus-pseudocamptodactyly syndrome (OMIM #158300), caused by mutation in fetal MyHC (*MYH8*);¹⁵ distal arthrogryposis (DA) type 1 (OMIM #108120), type 2A (OMIM # 193700; Freeman–Sheldon syndrome) and type 2B (OMIM # 601680; Sheldon–Hall syndrome), caused by mutations in embryonic MyHC (*MYH3*).^{16,17}

Here we describe for the first time the clinical findings, muscle morphology and molecular genetic characteristics in six patients from four unrelated families with recessive missense mutations in *MYH2* and also a patient with a novel recessive truncating *MYH2* mutation. The results support the concept that recessive missense mutations and truncating mutations as well as dominant missense mutations in *MYH2* may cause myopathy with ophthalmoplegia as an important clinical finding.

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MATERIALS AND METHODS

Patients

Seven patients from five unrelated families were clinically assessed, together with parents, when available. The study was approved by the regional ethics review board in Gothenburg, Sweden. The patients gave their informed consent.

Muscle morphology

Muscle biopsy was performed in five of the patients and subjected to routine enzyme histochemical analyses. In two patients (C and D), immunohistochemical analysis of MyHC isoforms was performed as previously described.¹⁸ In patient A, immunohistochemistry was performed for IIa and IIx fast MyHC isoforms (MY32, Sigma) and β -myosin (anti-slow myosin, Chemicon International, Melbourne, VIC, Australia).

DNA analysis

Genomic DNA was extracted from frozen skeletal muscle or peripheral blood using DNA Extraction Kit (Qiagen, Hilden, Germany). The entire coding region and exon–intron boundary regions of *MYH2* were analyzed by PCR and direct sequencing as previously described (*MYH2* sequence sources in GenBank: Genomic DNA accession number: NG_013014.1, GI: 260593654, mRNA Version: NM_017534.5, Gene ID: 4620, HGNC: 7572, Protein ID: Q9UKX2).¹⁹

Complementary DNA (cDNA) analysis

Total RNA was extracted from muscle tissue of the patients A, C and D using the Total RNA Isolation System (Promega, Madison, WI, USA). Synthesis of first-strand cDNA was performed using Ready-To-Go You-Prime First-Strand Beads (Amersham Pharmacia Biotech, Uppsala, Sweden) according to the manufacturer's instructions using 1 μ g total RNA.

To analyze the splicing of exon 4 of *MYH2* in patient A, PCR was performed on cDNA with two different primer pairs in order to specifically amplify fragments of *MYH2* cDNA. The first primer pair spans exon 1 to 10 as previously described¹¹ and the second primer pair was 5'-GGAGAAAAGTGA CGGTGAA-3' (forward primer, corresponding to nucleotide 169–188 of human MyHC IIa cDNA sequence; GenBank accession number: AF111784.1, GI: 4808812) combined with 5'-ATCTGTGGCCATCAGTTCTTCTCT-3' (backward primer, corresponding to nucleotide 986–1008 of human MyHC IIa cDNA). The resulting PCR products were analyzed by sequencing. Exons are numbered as in AF111784.1.

In addition, PCR was performed on cDNA with a primer pair covering exon 15 to 20 to analyze the p.Leu802Ter mutation in patient D as previously described.¹¹

To analyze the relative expression of different MyHC isoforms in muscle tissue at the transcript level, fragment analysis of multiplex PCR of cDNA using MyHC-specific primers was performed as previously described.¹⁸

RESULTS

Clinical findings

The clinical findings are summarized in Table 1.

Patient A was an 8-year-old boy of Sri Lankan descent who presented with external ophthalmoplegia at age 5 years. He complained of leg pains at night, helped by his parents sitting on his legs and feet, with no clear history of generalized weakness, fatigue, abnormal endurance, muscle cramps or exertional myalgia. He had mild weakness of shoulder abduction and neck flexion, almost complete external ophthalmoplegia, mild upper facial muscle weakness, mild ptosis and a mildly increased lumbar lordosis. His neurological examination was otherwise normal. His asymptomatic parents were non-consanguineous and had no muscle weakness on examination. His younger sister was clinically normal (Figure 1a). Serum creatine kinase (CK) and lactate/pyruvate levels were normal. Two common mitochondrial DNA mutations (m.8334A>G and

m.3243A>G; GenBank NC_012920.1) were absent in DNA from blood and urinary sediment.

Family B was a highly consanguineous Jewish family originating from Iran (Figure 1b). The proband (patient B1) was a 51-year-old woman with a very slowly progressive external ophthalmoplegia and mild facial and proximal limb muscle weakness probably beginning in late childhood. There was also very mild respiratory muscle weakness as assessed by a bedside spirometry. One of her two brothers (patient B2), and one of her two sisters (patient B3) were similarly clinically affected (Table 1). In addition, although both parents died before this family came to our attention, the available medical records document external ophthalmoplegia in the father at the age of 36 years, suggesting a homozygosity with a pseudodominant inheritance of an apparently autosomal recessive disease. The available testing in this family showed normal CK, electrocardiography and echocardiography. EMG showed borderline mixed findings.

Patient C had no relevant family history of neuromuscular disease and was of English ancestry (Figure 1c). There were no neonatal problems. Ophthalmoplegia, without ptosis, was noted at age 12 years. At age 43 years, he developed severe epilepsy, and mild proximal muscle weakness was noted. Examination showed generally thin musculature, severe ophthalmoplegia without ptosis, and mild facial, neck flexion and proximal limb weakness. There was some grip weakness and mild finger contractures. He was unable to rise from a low squat but could walk unaided; distal leg muscles were normal. His course was complicated by an episode of status epilepticus of unknown cause, but subsequently epilepsy was well controlled; brain MRI was unremarkable. CK was normal. Neurophysiology showed normal nerve conduction studies, normal neuromuscular transmission, and a myopathic EMG. He died unexpectedly and suddenly during sleep at the age of 45 years. Post mortem showed advanced coronary artery disease and cause of death was thought to be a cardiac arrhythmia.

Patient D was a Swedish man whose unaffected mother originated from Finland (Figure 1d). At age 57 years, he presented with near-complete ophthalmoplegia. He had noted slight slowness in running as a child, but was able to participate in several sport activities. Examination revealed slight weakness in neck flexion, elbow flexion and extension and hip flexion, and moderate weakness in abdominal muscles and handgrip. Cardiac function and forced vital capacity were normal. His serum CK was 1–2 $\times$ upper limit of normal.

Patient E was from the United Kingdom but of Indian descent. There was no family history of neuromuscular disease (Figure 1e). He presented with a very weak suck and was fed milk with a teaspoon for the first six weeks of life, after which he improved enough to be bottle fed. In childhood, he was good at sport. Ptosis developed in his twenties and proximal weakness developed in his forties. Examination at age 60 years showed ptosis and near complete ophthalmoplegia. Periocular weakness was more marked than perioral weakness. He had neck flexion and proximal weakness with preservation of distal muscles and could walk unaided. MRI of the thighs demonstrated prominent fatty infiltration in the quadriceps, adductors and semitendinosus muscles. There was relative sparing of the biceps femoris and sartorius muscles (Figure 2). CK was 3–4 $\times$ upper limit of normal.

Muscle pathology

The histopathological findings are summarized in Table 1.

In patient A, biopsies of the deltoid and quadriceps muscles revealed type 1 fiber uniformity (confirmed by immunohistochemistry) but otherwise no significant abnormalities (Figure 3). Analysis of MyHC transcripts also revealed expression of only *MYH7*

Table 1 Clinical findings, muscle pathology y and *MYH2* mutations

Patient	A	B1	B2	B3	C	D	E
M/F	M	F	M	F	M	M	M
Age at onset	5 years	Early childhood	Early childhood	Early childhood	12 years	Childhood	Neonatal
Delayed motor milestones	No Walked at age 13 months	Yes	Yes	Yes	No	No	No
Age at clinical investigation	6	51	43	40	44	57	60
Facial muscle weakness	Mild - upper facial muscles	Yes	Yes	Yes	Mild	No	Yes
Ptois	Mild bilateral	No	No	No	No	No	Marked
External ophthalmoplegia	Almost complete	Yes	Yes	Yes	Almost complete	Almost complete	Almost complete
Neck flexor muscle weakness	Mild	Mild	Mild	Mild	Moderate	Mild	Moderate
Proximal muscle weakness	Mild shoulder abduction	Mild to moderate	Mild to moderate	Mild to moderate	Mild	Mild abdominal, upper arms, hip flexion	Mild
Distal muscle weakness	Mild wasting of thenar and hypothenar eminences	Mild	Mild	Borderline	Grip weak, feet normal	Grip weak, feet normal	No
Joint contractures	No	No	No	No	Mild finger contractures	No	No
Scoliosis	Mild increase in lumbar lordosis	No	No	No	No	No	No
Age at muscle biopsy (years)	6	40			44	58	55
Investigated muscle	Deltoid and Quadriceps	Biceps	NI	NI	Deltoid	Deltoid	Quadriceps
Muscle pathology	Type 1 fiber uniformity	Type 1 fiber uniformity			Slight variability in fiber size, occasional fibers with internal nuclei, small type 2A fibers	Fatty infiltration, Fiber size variability, internal nuclei, type 1 fiber predominance	Fatty infiltration, Fiber size variability, internal nuclei, type 1 fiber uniformity
<i>MYH2</i> mutation (Homozygous or compound heterozygous)	c.533C>T (ACT>ATT)	c.706G>A (GCC>ACC)	c.706G>A (GCC>ACC)	c.706G>A (GCC>ACC)	c.1591T>C (ATG>ACG)	c.1331C>T (CGC>TGC) & c.2405T>A (TTG>TAG)	c.4352AdeI (AAG>AGC)
Predicted protein alteration	p.Thr178Ile	p.Ala236Thr	p.Ala236Thr	p.Ala236Thr	p.Met531Thr	p.Arg445Cys & p.Leu802Ter	p.Lys1451 SerfsTer40

Abbreviations: F, female; M, male; NI, not investigated

(Figure 4). In family B, biopsy of the biceps muscle of the proband showed poor fiber type differentiation but without more specific alterations. In patient C, a deltoid muscle biopsy showed increased variability of fiber size because of numerous small type 2A muscle fibers expressing MyHC IIa (Figure 5). In agreement with the protein analysis performed by immunohistochemistry, the analysis of MyHC transcripts showed presence of all three major isoforms with a reduced amount of MyHC IIa (Figure 4). Patient D demonstrated, in a biopsy of the deltoid muscle, fatty infiltration and extreme type 1 fiber predominance. Several of the type 1 fibers showed internalized nuclei and structural changes (Figure 6). Only two type 2B fibers expressing MyHC IIx as demonstrated by ATPase activity and immunohistochemistry were identified in the entire sample. No fibers expressing MyHC IIa were identified. In patient E, a quadriceps muscle biopsy showed fatty infiltration, fiber size variability, type 1 fiber uniformity and numerous fibers with internalized nuclei.

Molecular genetics

Five different single-nucleotide substitutions and one single-nucleotide deletion in *MYH2* were identified (Table 1).

In patient A, we identified a novel homozygous C to T change affecting the last highly conserved nucleotide of the 3' end of exon 4

(c.533C>T) (r.661c>u) predicted to result in a substitution of the polar uncharged threonine at position 178 to the nonpolar isoleucine (p.Thr178Ile) (Figure 1a). Sequencing of *MYH2* cDNA from skeletal muscle confirmed normal splicing of exons 4 and 5 (Figure 1a). Both unaffected parents of patient A were heterozygous for the c.533C>T mutation.

The three siblings from family B (B1, B2 and B3) were homozygous for a missense mutation (c.706G>A) in exon 6. Although the parents were not available for investigation, this indicates that the mutation was inherited from both parents. The mutation changes the conserved nonpolar amino-acid alanine at position 236 to the polar uncharged threonine p.(Ala236Thr) (Figure 1b).

Patient C was homozygous for a novel missense mutation (c.1591T>C) in exon 14, which changes the nonpolar amino-acid methionine at position 531 to the polar uncharged threonine (p.Met531Thr) (Figure 1c). The parents were not available for investigation.

In patient D, we identified two sequence variants. First, a novel heterozygous missense mutation (c.1331C>T) (r.1461c>u) in exon12, which changes the positively charged arginine at position 445 to the polar uncharged cysteine (p.Arg445Cys). The second variant was a heterozygous nonsense mutation (c.2405T>A)

Figure 1 Family pedigrees and sequence electropherograms of the *MYH2* mutations. The localization of mutations and amino-acid substitutions identified are depicted by arrows and red color, respectively. Filled symbols represent affected individuals, whereas unfilled symbols represent unaffected individuals. Double lines indicate a consanguineous union. The genotypes of each investigated family member at the *MYH2* are indicated. +, Mutant allele; -, wild-type allele. Arrows indicated the proband of each family. (a) Presence of the homozygous c.533C>T in the proband of family A changing the last highly conserved nucleotide of the 3' end of exon 4. The mutation results in a substitution of threonine at position 178 to isoleucine (p.T178I). Sequencing of *MYH2* cDNA from skeletal muscle confirmed normal splicing of exons 4 and 5. The stop and start of the exons are indicated by arrow. Both unaffected parents of patient A were heterozygous for the c.533C>T mutation. (b) Three affected siblings from the highly consanguineous family B (B1, B2 and B3) were homozygous for the c.706G>A missense mutation in exon 6, changing the conserved amino-acid alanine at position 236 to threonine (p.A236T). (c) Appearance of the homozygous c.1591T>C missense mutation in exon 14 in patient C changing the amino-acid methionine at position 531 to the threonine (p.M531T). The start of exon 14 is indicated by a arrow. (d) Presence of two variants in *MYH2* in patient D. First the heterozygous c.1331C>T missense mutation in exon 12 charging arginine at position 445 to cysteine (p.R445C). The second variant was the heterozygous c.2405T>A nonsense mutation in exon 19, changing leucine at position 802 to a stop codon (p.L802*), which was inherited from the unaffected mother. The unaffected brother was heterozygous for the c.1331C>T mutation (p.R445C) indicating that this mutation was inherited from the father. Sequence analysis of cDNA in the region covering exon 15 to exon 20 of *MYH2* demonstrated homozygosity for the wild-type c.[2405T] allele, indicating nonsense mediated mRNA decay of the c.[2405A] mutated allele. (e) Patient E was homozygous for the c.4352del single-nucleotide deletion in exon 29, shifting the reading frame and results in a premature stop codon (p.(L1451Serfs*40)). There are additional siblings who have not been clinical investigated.

(r.2533u>a), which changes leucine at position 802 to a stop codon (p.Leu802Ter) in exon 19 (Figure 1d). This variant was previously identified in two patients from Finland.¹¹ The unaffected mother had only the c.2405T>A mutation (p.Leu802Ter) and the unaffected brother was heterozygous for the c.1331C>T mutation (p.Arg445Cys) indicating that the c.1331C>T mutation was inherited from the father, who was not available for investigation. Sequence analysis of cDNA in the region covering exon 15 through exon 20 of *MYH2* demonstrated homozygosity for the wild-type c.[2405T] allele, indicating nonsense-mediated mRNA decay of the c.[2405T>A] mutated allele (Figure 1d).

Patient E was homozygous for a novel single-nucleotide deletion in exon 29 (c.4352del) that shifts the reading frame and results in a premature stop codon p.(Lys1451SerfsTer40) (Figure 1e). The parents were not available for investigation.

We used a public resource, the Exome Variant Server (NHLBI Exome Sequencing Project (ESP), Seattle, WA, USA (<http://evs.gs.washington.edu/EVS/>); accessed on June 2012), to identify frequent population variants in *MYH2*. The *MYH2* c.706G>A; (p.(Ala236Thr)) variant (rs147708782) has not been identified in

8596 alleles in the European American population but it has been found in 1/4405 alleles in the African American population. In addition, the p.Arg445His but not p.Arg445Cys variant has been identified in 2/8598 alleles in the European American population and in 0/4406 alleles in the African American population.

Sequence variants were novel changes submitted to the LOVD, Leiden Open Variation Database (http://grenada.lumc.nl/LSDB_list/lsdbs).

DISCUSSION

The first human skeletal myopathy associated with a mutation in MyHC was an autosomal dominant MyHC IIa myopathy, characterized by congenital joint contractures that resolved during early childhood, external ophthalmoplegia and predominantly proximal progressive muscle weakness. The morphological changes were mainly restricted to type 2A fibers, which were either absent or varied in size and showed structural changes.^{8,10,18} A later study of patients of three

Figure 2 Magnetic resonance imaging of patient E showing T1 weighted transverse images of the thighs. There is advanced fatty infiltration of the quadriceps, adductors and semitendinosus. There is relative preservation of the sartorius and biceps muscles.

Figure 4 Analysis of the relative expression of myosin heavy chain (MyHC) I, MyHC IIa and MyHC IIx messenger RNA based on reverse transcriptase-PCR. The size of the peaks reflects the amount of cDNA. Patient A express only MyHC I, whereas patient C express all three isoforms but unusually low amount of MyHC IIa.

Figure 3 Deltoid muscle of patient A demonstrating type 1 fiber uniformity but otherwise no major changes. (a) Hematoxylin and eosin. (b) Myofibrillar ATPase pH 9.34. (c) Immunofluorescence of slow myosin (MyHC I). (d) Immunofluorescence of fast myosin (MyHC IIa and IIx).

Figure 5 Deltoid muscle of patient C showing increased variability of fiber size with small type 2A fibers (arrows). (a) Hematoxylin and eosin. (b) NADH-tetrazolium reductase. (c–e) Immunohistochemical staining of MyHC isoforms on serial muscle sections. The MyHC IIa antibody recognizes to some extent also MyHC IIx.

Figure 6 Deltoid muscle of patient D demonstrating fatty infiltration, and numerous internalized nuclei. Some of the fibers show structural alterations with irregular intermyofibrillar networks. (a) Hematoxylin and eosin. (b) NADH-tetrazolium reductase.

unrelated families with a recessive myopathy, ophthalmoplegia and the absence of type 2A muscle fibers revealed that all patients were compound heterozygous for truncating mutations in *MYH2*.¹¹ More recently it was demonstrated that the affected individuals in a large family with recessive myopathy and ophthalmoplegia linked to chromosome 17 p13.1-p12 were homozygous for a frame shift mutation in *MYH2* because of a single-base deletion.²⁰

In this study, *MYH2* was considered as the plausible disease-causing gene because of the clinical findings of ophthalmoplegia in addition

to muscle biopsy findings of absence or abnormal type 2A muscle fibers. Four of the five novel identified mutations in this study were missense mutations (p.Thr178Ile, p.(Ala236Thr), p.Arg445Cys and p.Met531Thr), in contrast to the previously described recessive *MYH2* truncating mutations. These four mutated MyHC IIa residues are conserved in paralogous as well as in orthologous MyHC isoforms.

The *MYH2* p.Thr178Ile substitution identified in patient A has previously been identified in its paralog in embryonic MyHC (*MYH3*) as a cause of Freeman–Sheldon and Sheldon–Hall syndromes with

dominant mode of inheritance demonstrating the deleterious effect on myosin of this mutation.^{16,17}

Similarly, the c.769C>T (p.(Ala234Thr)) mutation in *MYH3*, which is paralogous to the *MYH2* p.(Ala236Thr) mutation found in patient B1, was demonstrated to cause Sheldon–Hall syndrome with dominant inheritance in a three-generation family.¹⁶

The p.Met531Thr mutation, which was identified in patient C, may impair contractile function as Met531 is located at the surface of the actin-binding site within a highly conserved α -helical structure.²¹ In slow/ β cardiac MyHC (*MYH7*), a missense mutation c.1680T>C (p.(Ser532Pro)) in this α -helical structure is associated with dilated cardiomyopathy with dominant inheritance.²² In addition, the abnormally small type 2A muscle fibers in patient C, support the concept of a myopathy caused by MyHC IIa myosin defect in analogy with findings in the dominant myosin IIa myopathy.¹⁸ The patient had also adult onset epilepsy, and died of ischemic heart disease, which was probably unrelated to the myopathy.

The mutated *MYH2* p.Arg445 residue in patient D is paralogous to p.Arg442 in slow/ β cardiac MyHC (*MYH7*). A hypothetical variant of this residue (p.Arg442His) has been reported to cause dilated cardiomyopathy, endocardial fibroelastosis and heart failure at a very early age,²³ demonstrating the importance of this amino acid for the function of myosin. This residue is located in a critical region of the myosin head, close to the nucleotide-binding pocket of the motor protein²¹ where several mutations in *MYH7* have been associated with cardiomyopathy.^{24–27} The other heterozygous mutation in patient D was a nonsense mutation (p.Leu802Ter). This mutation was inherited from the patient's mother who originated from Finland where this mutation has previously been identified.¹¹ The heterozygous p.Arg445Cys mutation alone was identified in the patient's unaffected brother, excluding that this mutation is dominant in nature.

Whereas the novel frameshift mutation found in patient E (p.(Lys1451SerfsTer40)) can be expected to be recessive in accordance with the findings in the previous reports,¹¹ it is intriguing that the four novel missense mutations seem to be recessive. The paralogs of the p.Thr178Ile and p.(Ala236Thr) mutations are dominant in embryonic MyHC (*MYH3*) where they cause distal arthrogryposis syndromes.^{16,17} One plausible explanation would be that embryonic MyHC is the predominant MyHC isoform during early development, which may cause high vulnerability to heterozygous mutations. Similarly, a missense mutation at residue p.Arg442 in slow/ β cardiac, paralogous to *MYH2* p.Arg445, is dominant. Nearly, all pathogenic *MYH7* mutations are dominant, which may be explained by the fact that slow/ β cardiac MyHC is the predominant isoform in the cardiac ventricles. The pathogenesis of these dominant *MYH7* and *MYH3* mutations could be either haploinsufficiency, incorporation of a functionally defective protein into sarcomeres, or other dominant negative effects.

MyHC IIa on the other hand is one of three MyHC isoforms in adult human limb muscles and therefore the other isoforms may, to some extent, compensate for a defect. Although the parents of our patients were not reported to have any signs or symptoms of myopathy, there may be subclinical changes that perhaps could be revealed by morphological analysis. Not all *MYH2* missense mutations are recessive as the c.2116G>A (p.(Glu706Lys)) mutation was reported to cause dominant myopathy.¹⁰ The severity of the myopathy was, however, related to the amount of expressed MyHC IIa in muscle, ranging from clinically mild in childhood and a more pronounced and progressive course with increased CK levels in adults.¹⁸

Inactivation of the genes encoding adult fast skeletal myosin heavy chain IIb and IId/x and creation of MyHC IIx and MyHC IIb null mice have indicated that these genes are required for the normal muscle development and function of adult skeletal muscle in the mouse.^{28–32} MyHC IId/x null mutants showed growth inhibition, muscle weakness and histological abnormalities with abnormal muscle fibers and increased interstitial connective tissue.³¹ There was a compensatory increase in MyHC IIa expression in the MyHC IId/x null mice. The MyHC IIb null mutants showed decreased number of muscle fibers and compensatory increase in MyHC IId/x and hypertrophy of the remaining fibers.²⁹ There were structural abnormalities of the muscle fibers and increased connective tissue in selective muscles. It was concluded from these studies that the different fast MyHC isoforms are functionally unique and cannot substitute for one another. MyHC IIa null mice have been reported but not characterized in detail.³³

The involvement of extraocular muscles in all patients with MyHC IIa myopathy further supports the notion that ophthalmoplegia is a clinical hallmark of patients with defects in *MYH2*. The ophthalmoplegia can be explained by the fact that MyHC IIa is a major isoform in extraocular muscle.^{34,35} Results from MRI of extraocular muscles in patients homozygous for truncating *MYH2* mutations have demonstrated atrophy with fatty infiltration²⁰ in accordance with skeletal muscle of the extremities as demonstrated in patient E (Figure 2).

Although our patients, in retrospect, had childhood onset symptoms, the relatively benign progression may cause presentation in adult years. Clinically, the combination of relatively mild and slowly progressive myopathy with ophthalmoplegia (with or without ptosis) should alert the physician to the possibility of *MYH2* mutations. In addition, morphological analysis of the muscle biopsy with demonstration of abnormally small 2A fibers or type 1 fiber uniformity would be further predictors of the probability of identifying a *MYH2* genetic cause.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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