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Homozygous Recessive MYH2 Mutation Mimicking Dominant MYH2 Associated Myopathy

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Abstract

Mutations in *MYH2* that encodes myosin heavy chain IIa cause both dominant and recessively inherited myopathies. Patients with dominantly inherited *MYH2* missense mutations present with ophthalmoplegia and progressive proximal limb weakness. Muscle biopsy reveals rimmed vacuoles and inclusions, prompting this entity to initially be described as hereditary inclusion body myopathy 3. In contrast, patients with recessive *MYH2* mutations have early onset, non-progressive, diffuse weakness and ophthalmoplegia. Muscle biopsy reveals near or complete absence of type 2A fibers with no vacuole or inclusion pathology. We describe a patient with childhood onset ophthalmoplegia, progressive proximal muscle weakness beginning in adolescence, and muscle biopsy with myopathic changes and rimmed vacuoles. Although this patient's disease course and histopathology is consistent with dominant *MYH2* mutations, whole exome sequencing revealed a c.737 G>A p.Arg246Thr homozygous *MYH2* variant. These findings expand the clinical and pathologic phenotype of recessive *MYH2* myopathies.

Keywords

MYH2; myosin heavy chain IIa; myosinopathy; genetics

Introduction

External ophthalmoplegia is seen in a wide variety of neuromuscular disorders including congenital myopathies, mitochondrial disorders, congenital myasthenic syndromes, and oculopharyngeal muscular dystrophy. A subset of patients with external ophthalmoplegia and variable degrees of skeletal muscle weakness have been found to harbor *MYH2* mutations.[1] Autosomal dominant (AD) mutations in *MYH2*, have classically presented with congenital joint contractures that resolve with time, adolescent onset external ophthalmoplegia, and progressive proximal weakness.[2,3] Muscle biopsy reveals dystrophic changes, rimmed vacuoles, and intranuclear and cytoplasmic inclusions leading this entity to initially be termed hereditary inclusion body myopathy HIBM3 <http://www.omim.org/entry/605637>. [2] Autosomal recessive (AR) mutations in *MYH2* also cause

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external ophthalmoplegia but distinct from AD associated *MYH2* myopathy, have mild, diffuse weakness that presents in early childhood and is largely non-progressive.[1,4–6] Unlike AD associated *MYH2* myopathy, patient muscle has small or absent type 2A fibers without vacuoles or protein inclusions.[4–6] In the present report we describe a family with a previously unreported c.737 G>A p.(Arg246Thr) homozygous *MYH2* variant.

Case Report

The patient is a 32-year-old male of northern Indian descent who was the child of consanguineous first cousin parents (Figure 1A). His birth was unremarkable and his early motor milestones were normal with the exception that he ran and jumped at a later age than his peers. Weakness became more pronounced in his 20s, when he developed progressive proximal weakness, dysphagia with solid food, and a weak cough. Notably his 21-year-old brother has similar but milder symptoms with difficulty climbing stairs (Figure 1A). Examination of the proband at age 32 revealed near complete ophthalmoplegia, mild eye and lip closure weakness, non-fatigable ptosis, nasal speech and mildly increased joint laxity without contractures. He had diffuse muscle atrophy that was most prominent in the quadriceps. Strength assessment was 5/5 and symmetric with the exception of 4/5 deltoid strength, 4/5 bicep strength, 4/5 triceps strength, 3/5 iliopsoas strength, 4/5 quadriceps strength and 4/5 hamstring strength. His gait was consistent with pelvic girdle weakness and he utilized a modified Gower to rise from the floor. Reflexes were 1+ throughout and sensation was intact to all modalities. Forced vital capacity was 75% of predicted. Serum creatine kinase ranged from 595 to 893 IU/L (normal 20–200 IU/L). Acetylcholine receptor antibodies were absent and lactate:pyruvate ratio was normal. Nerve conduction studies were normal. Electromyography revealed fibrillations, positive sharp waves and small units in deltoids and biceps suggesting an active myopathy. Ultrasound demonstrated increased signal in proximal arms and legs with selective involvement of the medial gastrocnemius. A biceps muscle biopsy (Figure 2) revealed marked fiber size variation with hypertrophied fibers as large as 280 microns in diameter, rimmed vacuoles, diffusely increased endomysial connective tissue, large pyknotic nuclear clumps, and altered internal architecture. ATPase stains poorly differentiated fiber types, but most large fibers were type 1 (Figure 2E). Immunofluorescent myosin stains (Figure 2E) demonstrated presence of MyHCIIa in primarily small fibers or larger fibers with abnormal internal architecture. Unlike control muscle, MyHCIIa expression often co-existed with MyHCI in type 2 fibers from the proband's muscle. Mitochondrial enzyme assays were normal.

Exome sequencing was performed using Agilent SureSelect Human All Exon V2 according to the manufacturers protocol. We used Genesis 2.0, a web based collaborative genome analysis tool and variants were filtered for non-synonymous splice site, nonsense, or small insertion or deletion variants. We used a strict criterion filter to identify homozygous, compound heterozygous, or splice site variants with a recessive pattern of inheritance previously associated with muscle disease and a MAF <0.001 within the ExAC browser. This resulted in identification of 1 homozygous variant in *MYH2* (MAF 0.000082, c.737 G>A, NM_017534.5). Direct sequencing confirmed the homozygous c.737 G>A, p.R246H change in *MYH2* in the proband and heterozygous changes in both parents (Figure 1B). This amino acid is highly conserved among lower species (Figure 1C) with phastCons score of 1

and phyloP score of 5.664. The mutation is predicted to be damaging by *in silico* analysis tools SIFT, Mutation Taster, and Polyphen2. This amino acid is also conserved between myosin isoforms. Paralogous mutations in *MYH7* (p.R243H) have been associated with dominant cardiomyopathies.[7,8]

Discussion

MYH2 myosinopathies are associated with an autosomal recessive or dominant pattern of inheritance with distinct pathologies and phenotypes. We believe the homozygous *MYH2* c.737 G>A p.(R246H) variant is pathogenic based on similar phenotypes seen in *MYH2* myosinopathy patients, segregation of disease state with homozygosity, and rarity of this variant within the population. Pathogenicity is further supported by our investigations showing this amino acid is conserved in lower species and different myosin isoforms, our *in silico* analysis predicting the change to be pathogenic, and paralogous mutations in *MYH7* are associated with cardiomyopathy.[7,8] Our patient with a homozygous missense variant in *MYH2* demonstrates a majority of clinicopathological characteristics seen in patients with autosomal dominant mutations and few attributes typical of patients with autosomal recessive mutations (Figure 3A).[1–5,9]

Several patients with AD *MYH2* mutations have been reported with phenotypes deviating from the norm.[10,11] These patients instead have a phenotype and pathologic features more consistent with the AR form. The majority of recessive *MYH2* mutations result in premature stop codons or affect the myosin motor domain (Figure 3). They are thought to cause disease through reduction or absence of MyHCIIa expression, or loss of function due to involvement of the myosin motor domain.[4,6] The majority of AD mutations involve the coiled coil rod domain (Figure 3) and are thought to exert a dominant negative effect by altering proper assembly of myosin homodimers.[10,11] The E706K mutation impacts the SH1 helix critical for the lever arm swing and is thought to cause a dominant negative effect on muscle via perturbation of conformational changes during ATP hydrolysis.[12]

The proband's c.737 G>A p.(R246H) *MYH2* mutation affects the myosin motor domain, similar to other AR patients, but phenotypically resembles an AD patient with progressive proximal weakness and rimmed vacuoles on biopsy. The patient's muscle biopsy demonstrates MyHCIIa expression on immunohistochemistry, suggesting disease pathogenesis may be different from typical AR patients with absent MyHCIIa transcript expression.[4] Reduced MyHCIIa transcript expression, however, remains a mechanistic possibility. It is interesting to note MyHCIIa is primarily found within small fibers or those with an irregular myofibrillar staining pattern, supporting that disease pathogenesis may be related to impaired MyHCIIa function. The patient's hybrid muscle fibers expressing multiple MyHC combinations has been reported with AD *MYH2* mutations.[12] The combinations of fast and slow MyHCs within single fibers explains the poor fiber type differentiation with ATPase and highlights the importance of specific myosin isoform staining.

While it is unusual that our patient's homozygous *MYH2* mutation is associated with vacuoles, similar situations have been described with AR mutations in *MYOT* and *DES*.

[13,14] Although we speculate that the p.R246H mutation manifest in the homozygous state, it is conceivable that it manifests sub-clinically when heterozygous. While the mechanism for this patient's phenotypic deviation from other AR *MYH2* patients remains unclear, the present study expands the current range of disease causing mutations of *MYH2* as well as the phenotype associated with AR *MYH2* mutations.

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Figure 1.

Family tree of proband (black), and brother (gray) with reported similar symptoms, and consanguineous first cousin parents (A). Sequence tracing demonstrating patient's c.737 G>A mutation (B) and heterozygous mother and father. Conservation at p.R246 in vertebrates (C).

Figure 2.

Muscle biopsy hematoxylin and eosin (H&E) stain demonstrating chronic myopathic changes with significant fiber size variability, increased endomysial connective tissue, large pyknotic nuclear clumps (A). Rimmed vacuoles are demonstrated on H&E (B) and trichrome stains (C). Serial sections of control and patient muscle stained with ATPase pH 9.4, 4.6, and 4.3 or immunofluorescence stains with collagen IV (red), and MyHC (green) (D). Collagen IV was stained with Millipore AB748. MyHC stains used monoclonal antibodies from the Iowa Hybridoma bank: MyHCIIa (SC-71) and MyHCIIx (6H1). MyHC1 was stained with Abcam [NOQ7.5.4D] (ab11083). Examples of different fiber types and associated myosin stains are labeled for control and patient tissue. Poor fiber type differentiation on ATPase staining is noted in patient tissue and does not correlate with myosin stains. Type 1 fibers express MyHC1 in control tissue. Patient fibers expressing only MyHC1 have discordant dark staining on ATPase 9.4 (*). Type 2A fibers contain only

MyHCIIa myosin in control tissue. No true 2A fibers are present in patient tissue. Type 2B fibers contain MyHCIIa and MyHCIIx in control tissue, whereas patient tissue has many hybrid fibers with variable expression of MyHCIIa, MyHCIIx, and MyHC1 (arrows). Scale bars indicate 100 microns.

A	Autosomal Recessive	Autosomal Dominant	Proband
Age at onset	Weakness - Childhood	Congenital contractures. Adult onset weakness.	Weakness - Adolescence
Disease Course	Non-progressive	Progressive	Progressive
Ophthalmoplegia	Present	Present	Present
Facial weakness	Present	Infrequent	Mild
Contractures at birth	Absent	Present	Absent
Weakness pattern	Proximal & distal	Proximal > distal Legs > Arms	Proximal > distal Legs > Arms
Scoliosis	Frequent	Infrequent	Absent
CK (IU/L) Normal 50-300	Normal to 4x	2-10x normal in adults with progressive disease	595-893
Muscle Histopathology	Myopathic changes with fatty infiltration. Type 1 predominance or uniformity with reduced or absent MyHCIIa and 2A fibers. Core like disruption of myofibrillar network.	Myopathic and dystrophic changes with increased connective tissue and fat. Rimmed vacuoles. MyHCIIa present. Reduced number and small type 2A fibers. Inclusions of 15-21-nm filaments.	Myopathic and dystrophic changes with increased connective tissue. Rimmed vacuoles. MyHCIIa present in small fibers or larger fibers with abnormal internal architecture.

Figure 3.

Table summarizing key clinical characteristics seen in patients with AR or AD *MYH2* mutations (A). [3,4,6,10,11,15–17] Mutation map for AR and AD *MYH2* mutations. Bolded mutation indicates proband (B).