**MYH1 is a candidate gene for recurrent rhabdomyolysis in humans**

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ABSTRACT

Rhabdomyolysis is a serious medical condition characterized by muscle injury and there are recognized genetic causes especially in recurrent forms. The majority of these cases, however, remain unexplained. Here, we describe a patient with recurrent rhabdomyolysis in whom extensive clinical testing failed to identify a likely etiology. Whole-exome sequencing revealed a novel missense variant in \textit{MYH1}, which encodes a major adult muscle fiber protein. Structural biology analysis revealed that the mutated residue is extremely well conserved and is located in the actin binding cleft. Furthermore, immediately adjacent mutations in that cleft in other myosins are pathogenic in humans. Our results are consistent with the finding that \textit{MYH1} is mutated in rhabdomyolysis in horses and suggest that this gene should be investigated in cases with recurrent rhabdomyolysis.
INTRODUCTION

Rhabdomyolysis is a medical condition in which acute muscle injury triggers a cascade of pathophysiological changes with potentially life-threatening complications (Zutt et al., 2014). The majority of cases are acquired secondary to trauma, infection or drug reactions. However, single genetic causes of rhabdomyolysis must be considered especially in cases with recurrent rhabdomyolysis. Although several genes were implicated, the majority of cases remain without genetic diagnosis even after whole-exome sequencing (WES) (Vivante et al., 2017). This frequent absence of molecular diagnosis highlights the need to identify additional genetic forms of recurrent rhabdomyolysis. We have previously leveraged the special population structure of Saudi Arabia to characterize a novel recessive form of recurrent rhabdomyolysis. This form was caused by a specific variant in KCNJ11 that resulted in neonatal hyperinsulinism and life-long recurrent rhabdomyolysis (Albaqumi et al., 2014). In continuation of that effort, we describe here a potentially novel recessive form of recurrent rhabdomyolysis, which we propose to be caused by MYH1 mutation.

CLINICAL REPORT

The index is 18 years old male who is the only child of healthy Saudi consanguineous parents and a product of in-vitro fertilization (IVF). He presented with history of recurrent episodes of muscle weakness and rhabdomyolysis after upper respiratory infection. His history started around 4 years ago when he had his first attack of severe muscle pain, cramps, weakness and discoloration of his urine one week following upper respiratory infection. He was evaluated and diagnosed with viral rhabdomyolysis. He had multiple attacks after that incident with a frequency of 1-2 per year with the highest recorded CK > 40,000 U/L. He is otherwise healthy except for atopic dermatitis. He is not using any drugs and had no exposure to toxins.
Furthermore, there was no history of seizures, loss of consciousness, trauma, abdominal pain, bulbar symptoms, sphincter abnormalities, or sensory or cerebellar manifestations. EMG showed evidence of proximal and mild irritable myopathic changes. MRI revealed nonspecific diffuse abnormal bone marrow signal intensity with questionable multifocal small epiphyseal bone marrow infarcts, but no evidence of muscular pathology. The patient underwent a very extensive work up for rhabdomyolysis. Electromyography performed a few weeks after one episode showed some myopathic motor unit potentials with rare fibrillation potentials and positive sharp waves. Complete blood count, bilirubin, uric acid, kidney function, liver function, serum electrolytes, fasting acylcarnitine profile, lactate, pyruvate, endocrine and autoimmune markers were not revealing.

A commercial next generation sequencing of 47 genes known for their association with rhabdomyolysis and metabolic myopathies and later whole mitochondrial genome and whole exome sequencing did not identify any clinically significant alteration.

A biopsy of the left vastus lateralis was obtained a few months after one episode of rhabdomyolysis (Figure 1). The routine hematoxylin-eosin (H&E) stain on the freshly frozen which showed occasional atrophic fibers, most of which having polygonal contours, while a minority were angular. Scattered pyknotic nuclear clumps were also noted. Occasional mildly hypertrophic fibers were also seen. Some of the atrophic fibers were darkly stained with the nicotine amide dehydrogenase-tetrazolium reductase (NADH-TR) and the nonspecific esterase. One necrotic fiber was noted in the entire biopsy. Interstitial connective tissue was unremarkable. Other mild changes included occasional split fibers and moth-eaten fibers on oxidative enzymes, i.e. NADH-TR and succinic dehydrogenase (SDH). The latter also revealed mild subsarcolemmal mitochondrial proliferation; there were no frank “ragged blue” or ragged
red fibers. Transmission electron microscopy confirmed the presence of excess mitochondria and fat droplets without evidence of structural abnormality. Myofiber typing was assessed by immunohistochemistry using antibodies for the slow (clone WB-MHCs; Vector Laboratories, Burlingame, CA) and fast slow (clone WB-MHCf; Vector Laboratories, Burlingame, CA) isoforms of the myosin to detect type 1 and type 2 fibers, respectively. The ratio of type 1 to type 2 fibers was about 2:3. Atrophic fibers belonged to both types. There was no definite fiber type grouping.

**GENETIC AND STRUCTURAL BIOLOGY STUDIES**

In view of the negative clinical WES result, we enrolled the patient and both parents in an IRB-approved research protocol with informed consent (KFSHRC RAC#212053). Reanalysis of the WES file in conjunction with autozygome analysis as described before (Monies et al., 2017) revealed a novel homozygous variant in *MYH1*:NM_005963.4:exon14:c.1295A>C:p.K432T. This variant is absent in 2,379 local exomes and there is only one heterozygotes individual on gnomAD. It is predicted to be deleterious by PolyPhen (0.963), SIFT (0) and CADD (24.7). The lysine at position 432 is absolutely conserved across all species examined. Staining pattern for the MYH1 protein on muscle biopsy (cat:ab51263) was identical to that of fast MHC (Figure 1).

Therefore, we investigated its potential pathogenicity using a structural biology approach. *MYH1* encodes a cytoskeletal molecular motor that generates force and movement when interacting with actin. The generation of force is powered by the hydrolysis of ATP and release of the hydrolysis products (inorganic phosphate and MgADP), which is coupled to structural changes (Houdusse and Sweeney, 2016). The structure of MYH1 could be modeled with good confidence based on the crystal structure of the bovine beta-cardiac myosin in the post-rigor state.
Lys432 is part of the U50 subdomain of MYH1 and is located in the actin binding cleft. This cleft separates the upper 50kDa (U50) and lower 50kDa (L50) subdomains of the myosin motor (Figure 2). The cleft closes after binding to actin and forms a strong actin-binding interface, which includes residues from both subdomains. Lys432 and the residues in the opposing “strut” segment are highly conserved (Ashkenazy et al., 2016), and it is possible that they maintain stabilizing interactions while the cleft is closed in the rigor state of the motor. The substitution by the uncharged threonine would introduce an uncompensated negative charge in the cleft, and possibly affect the cleft closure during the power stroke. Robert-Paganin et al. (Robert-Paganin et al., 2018) used the three-dimensional structure of the bovine beta-cardiac myosin to study a set of 178 pathogenic variants, previously described as triggering a hypertrophic cardiomyopathic phenotype. The variants were found in all of the subdomains of the myosin heavy chain and they had a wide variety of effects, from altering the states and transitions populated during the power stroke, to significantly compromising the motor function and its stability. Four of these mutations target the immediate vicinity of Lys432 (Gly428Arg, Ala429Thr, Ala431Val, and Ala433Glu; the numbering corresponds to the human protein) (Figure 2). According to the authors’ in silico analysis, these mutations mostly either create steric clashes in the U50 core that might affect the protein’s stability and/or function, or target the U50 cleft in a way that affects the performance of the power stroke and hence protein function.

**DISCUSSION**

MYH1 encodes one of the four postnatal myosin heavy chain isoforms of the sarcomeric (contractile) myosin heterohexamer (Wang et al., 2017). In horses, it has been found that a
missense variant (Glu321Gly) is strongly associated with non-exertional rhabdomyolysis phenotype (Valberg et al., 2018). Interestingly, an earlier study in horses suggested that the same variant is associated with an immune-mediated myositis (Finno et al., 2018). However, Valberg and colleagues showed that rhabdomyolysis is not associated with evidence of lymphocyte infiltrates in their cohort, which would be consistent with the pattern of rhabdomyolysis observed in our patient (Valberg et al., 2018).

MYH1 has not been linked to human diseases thus far. Several lines of evidence support the hypothesis proposed in this report i.e. mutation of MYH1 causes recurrent rhabdomyolysis. First, the gene encodes a highly conserved component of the skeletal muscle contractile apparatus. Second, the horse ortholog has been implicated in the etiology of rhabdomyolysis (see above). Third, no alternative etiology of recurrent rhabdomyolysis was identified in the study patient despite extensive investigations. Fourth, the mutated residue corresponds to a stretch of highly conserved residues mutation of which has been documented to cause hereditary forms of muscle disease in humans. Nonetheless, we emphasize that future cases will be needed to confirm the proposed association between MYH1 and rhabdomyolysis in humans.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.
REFERENCES


FIGURE LEGENDS

Figure 1. Clinical features of patient with recurrent rhabdomyolysis and a novel MYHI variant

(A) Pedigree of the family.

(B) Level of creatine kinase (CK) at different times from the first visit. (*) indicate first episode of remarkably high CK level >40,000 U/L after upper respiratory infections. (**) indicate second episode of high CK level >25000 U/L after a heavy workout. (*** indicate the third episode of high CK level = 6336 with no clear trigger. Normal range (24-195 U/L). Note: not all the CK levels were recorded because the patient was following up at many different hospitals.

(C) Muscle biopsy of the vastus lateralis showing atrophic fibers with polygonal contours (a), angular severely atrophic fibers (open arrow heads) and pyknotic nuclear clumps (solid arrows) Hyper trophic fibers (h) are also seen. The only necrotic fiber with myophagocytosis is shown in the inset. (H&E, original magnification x400)

(D) SDH reaction depicts fibers with subsarcolemmal proliferation in the center of the image. Surrounding fibers show some pallor indicating mild disruption of the intermyofibrillar network (“moth-eaten” fibers). (Succinic dehydrogenase, original magnification x400)

(E) An angular fiber is positively stained with nonspecific esterase (open arrow head). (nonspecific esterase, original magnification x400).

(F) Type 1 fibers are positively stained with the slow MHC. (sMHC, original magnification x100)

(G) The fast MHC show reciprocal staining of type 2 fibers. (fMHC, original magnification x100)
(H) Normal staining is observed with the MHY1 antibody in a distribution corresponding to type 2 fibers. (MYH1, original magnification x100).

**Figure 2.** Investigation of the pathogenicity of *MYH1* missense variant using a structural biology approach

(A) Sequence chromatogram of *MYH1* variant.

(B) Homology model of MYH1. Lys432 (orange sticks; all labels are using the one-letter amino acid code) is located in the actin binding cleft (marked with a black triangle), which separates the U50 (dark blue) and L50 (light blue) subdomains of the myosin motor. The substitution of the negatively-charged lysine by the shorter and polar threonine might disrupt stabilizing interactions between Lys432 and the strut segment (teal sticks) and surrounding residues, potentially affecting the cleft closure during the powerstroke. The four mutations studied by Paganin et al. (5) that are located in the vicinity of Lys432, are shown as blue sticks.


(D) Multiple sequence alignment of Lys432Thr across different species showing the highly conservation of Lysine at position 432, Genomic Evolutionary Rate Profiling (GERP) score is 5.73. Protein sequence references are NP_005954.3, XP_003953177.1, NP_109604.1, NP_776542.1 and NP_001001244.1.
Figure 1

A  
I:1  I:2  II:1

B  
CK level (IU/L) vs Time from first visit (Months)

C  D  E

G  H

50x38mm (300 x 300 DPI)
Figure 2

A

B

C

D

For Peer Review

50x38mm (300 x 300 DPI)