The association of the Arg1277Gln mutation in the MYH7 gene with myosin storage myopathy in a Chinese family

Li-li Li
Qingdao University
Riu Li
Qingdao University
Dongyun Liu (sherolily920@163.com)
The Affiliated Hospital University Of QingDao University https://orcid.org/0000-0001-9852-8847

Research

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Abstract

Background Myosin storage myopathy (MSM) is caused by missense mutations in the MYH7 gene, which encodes the β-cardiac/slow skeletal muscle myosin heavy chain rod (MyHCI). MSM is an autosomal dominant/recessive myopathy characterized by subsarcolemmal accumulations of myosin in type I muscle fibers that results in weakness of the scapula, limb and distal muscles.

Methods Here, we report a MSM phenotype that was present across three generations of individuals from the same family, one of whom was a neonate.

Results At birth, the neonate had an elevated creatine kinase level and decreased muscle tone in the limbs. At 2 months of age, the infant's cervical vertebrae caused his head to be skewed to the right. At 7 months of age, the infant's development was delayed.Whole exome sequencing showed a novel heterozygous variant NM_000257.3: c.3830G>A (p.Arg1277Gln) at exon 28 of the MYH7 gene in the DNA of the infant and his father.

Conclusions Previously, which site has only been reported in 2 cases of cardiomyopathy; therefore, this study expands our knowledge of the clinical phenotypes associated with mutations within the rod region of MyHCI. Importantly, close follow-up of the neonate will provide important information on the natural history of MSM associated with MYH7 gene mutation.

Background

Myosin storage myopathy (MSM) is a rare congenital myopathy characterized by subsarcolemmal accumulations of myosin in type I muscle fibers. MSM is caused by missense mutations in the MYH7 gene, which encodes the β-cardiac/slow skeletal muscle myosin heavy chain rod (MyHCI) (1).

The MYH7 gene is approximately 22883 bp long, consists of 40 exons, and is located on chromosome 14. The MYH7 gene is one member of the MYH gene family, which encode the myosin heavy chains (MyHC) that comprise part of each class II myosin molecule (2), Mutations in the MYH7 gene can cause cardiomyopathies (3), such as hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), and left ventricular non-compaction cardiomyopathy (LVNC) (4–6),Mutations in the MYH7 gene are also common causes of hereditary skeletal muscle diseases, including myosin storage myopathy (MSM) and Laing Distal Myopathy (LDM) (7).

Here, we report a MSM phenotype associated with a mutation in exon 28 of the MYH7 gene that was present across three generations of individuals from the same family. This finding will widen the genotypic and phenotypic spectrum of MSM.

Methods

Patient 1 was a male neonate (gestational age 38 weeks and 6 days) born by cesarean section. Apgar scores at 1, 5, and 10 minutes were 10. The patient weighed 2180 g at birth, head circumference was 34 cm, and birth length was 47 cm. The birth was considered 'low birth weight' (BW<2500 g) and 'intrauterine growth restriction' (weight < 10th percentile); therefore, the infant was admitted to a neonatal intensive care unit. Physical examination showed the infant's neck was slightly shorter and the muscle tension of the limbs was slightly lower than normal; however, there were no other clinically significant changes in the skeletal muscle at this time. A myocardial enzyme test revealed high levels of creatine kinase (2317.00 U/L; normal 50-310 U/L), creatine kinase isoenzyme (59.00 U/L; normal 0-17 U/L), and lactate dehydrogenase (579.0U/L; normal 120-250U/L). Cardiac ultrasound examination demonstrated a patent foramen ovale but no cardiac hypertrophy. MRI examination of the brain was unremarkable. At 2 months of age, the infant's cervical vertebrae caused his head to be skewed to the right (**Figure 1A**), and muscle tension of the limbs was slightly lower than normal. During follow-up, the infant's growth and development were delayed. At 7 months of age, the infant's weight was < 10th percentile, body length was on the 3rd percentile, and head circumference was approaching the 10th percentile, having been on the 25^{th} percentile after the birth (**Table 1**). The infant failed to meet developmental milestones as he could not sit alone at 7 months of age.

Patient 2 was a 32 year old male and the father of Patient 1. Patient 2 developed scoliosis and kyphosis during childhood (the male of **Fig. 1 B or Fig. 1C**), but there were no clinical signs typical of LDM in adulthood, such as flexor muscle weakness, calf hypertrophy, or the inability to lift the first toe. The patient's height (153 cm) was shorter than normal, and he had an atypical gait. Cardiac ultrasonography showed no ventricular hypertrophy.

Patient 3 was Patient 2's mother. Patient 3 had the same phenotype as Patient1 and 2, including progressive scoliosis, a hunchback, and difficulty walking during childhood(the female of **Fig. 1C**). Patient 3 did not consent to genetic testing. Information on other family members was not available.

Whole genome sequencing

Whole genome sequencing was performed using the second-generation high-throughput method. 2 ml peripheral venous blood was sampled from each patient into an ethylenediaminetetraacetic acid (EDTA) anticoagulant tube. Genomic DNA was extracted using the QIAamp DNA Extraction Kit (QIAGEN), according to the manufacturer's instructions. DNA concentration was estimated by measuring the absorbance at 260 nm. DNA was extracted using magnetic beads. PCR amplification was performed followed by ligation with specific linkers. Gene regions were captured with two TruSight One Sequencing Panels (illumina Inc, USA) and amplified by PCR. The exons of 4811 clinically relevant genes were sequenced using a MiSeq sequencer (illumina Inc, USA).

Data were aligned on the UCSC hg19 reference sequence using the BWA algorithm (8) and annotated with published literature (9). Pathogenicity of mutations was predicted using clinical data and

bioinformatics software (PolyPhen2, LRT, Mutation Taster). Potential candidate mutations were obtained by analyzing the function, variation, and inheritance pattern of each gene.

Candidate mutation sequences were validated by Sanger sequencing. Primer sequences for PCR were Sense: 5'-GACCAGATGAATGAGCACCG-3'; Antisense:5'-AGGGCGTTCTTCGCCTTAAC-3'. After obtaining informed consent, whole exome sequencing was performed on the patients to identify novel variants residing in the *MYH7* gene.

Results

Whole exome sequencing showed a novel heterozygous variant NM_000257.3: c.3830G > A (p.Arg1277Gln) at exon 28 of the MYH7 gene in the DNA of Patient 1 and Patient 2 (Fig. 2). Patient 1's mother did not carry this variant, suggesting Patient 1 inherited the mutation from his father. This missense single nucleotide variant results in an amino acid substitution from Arg to Gln at position 1277 of the encoded protein. It has been reported in patients with hypertrophic cardiomyopathy (10), implicated in congenital myopathy, and is registered in the dbSNP147 database (rs397516195), but not in the ESP6500siv2_ALL, 1000 human genome (1000g2015aug_ALL) database. These findings, together with results from our bioinformatics analyses, suggest that this missense single nucleotide variant dictated the clinical phenotype seen in Patients 1, 2, and 3. The patients' did not provide informed consent for muscle biopsy or muscle MRI; therefore, there is a lack of additional data to support these findings.

Discussion

The MYH7 gene encodes MyHCI. It is composed of 40 exons, of which exons 3–21 encode the globular head region, including the head and neck of MyHCI, and exons 22–40 encode the rod region, including the hinge and light meromyosin chain (11). Clinical phenotypes associated with MYH7 gene mutations are diverse and dependent on the affected residue. Mutations causing defects of the head or proximal rod region of the myosin molecule are most likely to cause cardiomyopathy (9, 12, 13), and mutations of the distal rod region are associated with skeletal myopathy (11, 14). Mutations in exon 37–39 of the MYH7 gene are primarily responsible for MSM, while mutations in exon 32–36 result in LDM (15).

In the present study, we report a novel variant NM_000257.3: c.3830G > A in exon 28 of the MYH7 gene resulting in a single amino acid change p.Arg1277Gln in the rod region of MyHCI, damaging the protein structure of the myosin molecules in skeletal muscle. The individuals in this study had an MSM phenotype that was present across three generations from the same family. Previously, mutation at this site has only been reported in 2 cases of cardiomyopathy (10); therefore, the present study expands our knowledge of the clinical phenotypes associated with mutations within the rod region of MyHCI.

Clinically, MSM is characterized by hypertrophy of the limb muscles, the shoulder armor or the distal muscles (16, 17). Patients with mild clinical symptoms may present with elevated creatine kinase levels but no muscle involvement. Patients with severe clinical symptoms may experience skeletal muscle

involvement leading to movement disorders. Mutations in the MYH7 gene can also result in spinal deformities and respiratory muscle involvement, requiring surgical intervention (18), and there may be phenotypic overlap between skeletal myopathy and cardiomyopathy (19, 20). In the present study, Patient 2 presented with scoliosis and no involvement of the proximal, shoulder, or neck muscles. Patient 1, who was a neonate, had an elevated creatine kinase level and decreased muscle tone in the limbs at birth, the infant's cervical vertebrae caused his head to be skewed to the right at 2 months of age, and the infant's development was delayed at 7 months of age. To the authors' knowledge, this is the first report to document these clinical manifestations in association with the missense mutation NM_000257.3: c.3830G > A in exon 28 of the MYH7 gene (10).

The pathophysiological mechanisms that underlie MSM phenotypes resulting from MYH7 mutations remain to be elucidated (21). A drosophila MSM model was used to investigate the effects of L1793P, R1845W, and E1883K MSM mutant myosins expressed in an indirect flight and jump muscle myosin null background. Jump and flight ability were highly compromised in mutant animals. Indirect flight muscle structure showed myofibrillar disarray and degeneration with hyaline-like inclusions. Mutant myosin had a reduced ability to polymerize and decreased stability (22). Cultured human muscle cells were used to assess the impact of four mutation sites (L1793P, R1845W, E1883K and H1901L) on myosin assembly and muscle function and explore the mechanisms leading to protein aggregation in MSM. Findings showed that R1845W and H1901L mutants were prone to formation of myosin aggregates without assembly into striated sarcomeric thick filaments (23). Taken together, these data suggest that changes in the structural properties of slow/β-cardiac MyHCI due to mutation of the MYH7 gene may represent the primary trigger of MSM (23). Further studies are required to elucidate the pathogenic basis of MSM, which may inform clinical decision making and strategies for early diagnosis and treatment of the disease.

Conclusions

In summary, we report on three generations of individuals from the same family with an MSM phenotype and a novel variant NM_000257.3: c.3830G > A in exon 28 of the MYH7 gene. Previously, mutation at this site has only been reported in 2 cases of cardiomyopathy (10); therefore, the present study expands our knowledge of the clinical phenotypes associated with mutations within the rod region of MyHCI. Importantly, one of the patients in this study was a neonate. Close follow-up of this patient will provide information on the natural history of MSM associated with this mutation in the MYH7 gene.

Abbreviations

MSM
myosin storage myopathy
MyHCI
β-cardiac/slow skeletal muscle myosin heavy chain rod
HCM

hypertrophic cardiomyopathy
DCM
dilated cardiomyopathy
LVNC
left ventricular non-compaction cardiomyopathy
LDM
Laing Distal Myopathy
EDTA
ethylenediaminetetraacetic acid
QIAGEN
QIAamp DNA Extraction Kit

Declarations

Ethics approval and consent to participate

This study was approved by the ethics committee of The Affiliated Hospital of Qingdao University. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Consent for publication

All data published here are under the consent for publication.

Availability of data and materials

The datasets generated and analyzed during the present study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no conflict of interest.

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There was no financial assistance to support the study.

Authors' contributions

Lili Li and Dongyun Liu designed most of the investigation, data analysis and wrote the manuscript; Rui Li contributed to interpretation of the data and analyses. All of the authors have read and approved the manuscript.

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Table 1

Table 1: Patient 1: Growth rate

Months	0	1.5	2.4	2.8	3.6	4.5	5.1	6.5	7.9
Head circumference(cm)	34	36.5	38	38.5	39.5	40.5	41	42	42.5
Length(cm)	47	50.5	55	56	56	58	61	64.5	67
Weight(kg)	2.14	3.92	4.8	5.11	5.68	6.1	6.4	7.05	7.55

Figures

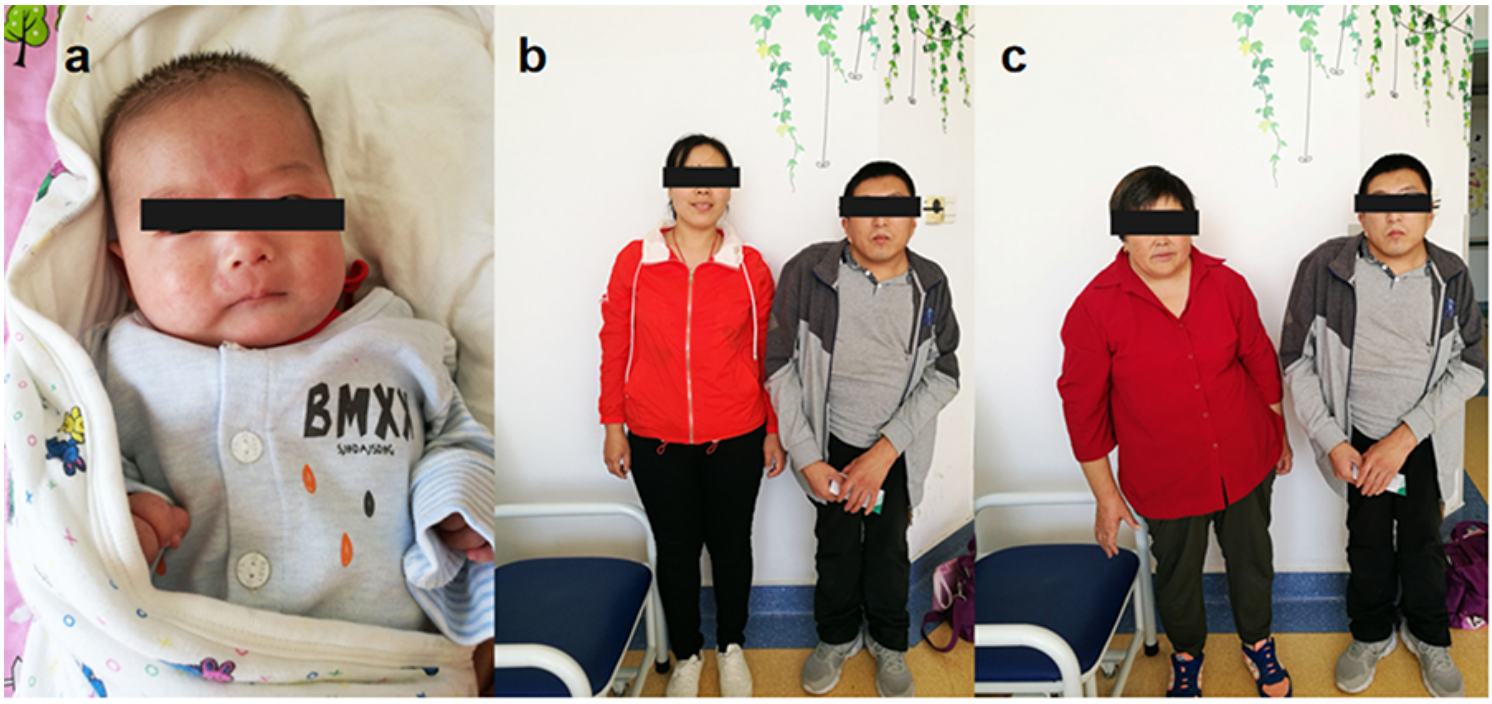

Figure 1

The MSM phenotype was present across three generations of individuals from the same family A) Patient 1: 2 months of age; B) Right: Patient 2 (Patient 1's father); Left: Patient 1's mother; C) Patient 2 and his mother (Patient 1's grandmother)

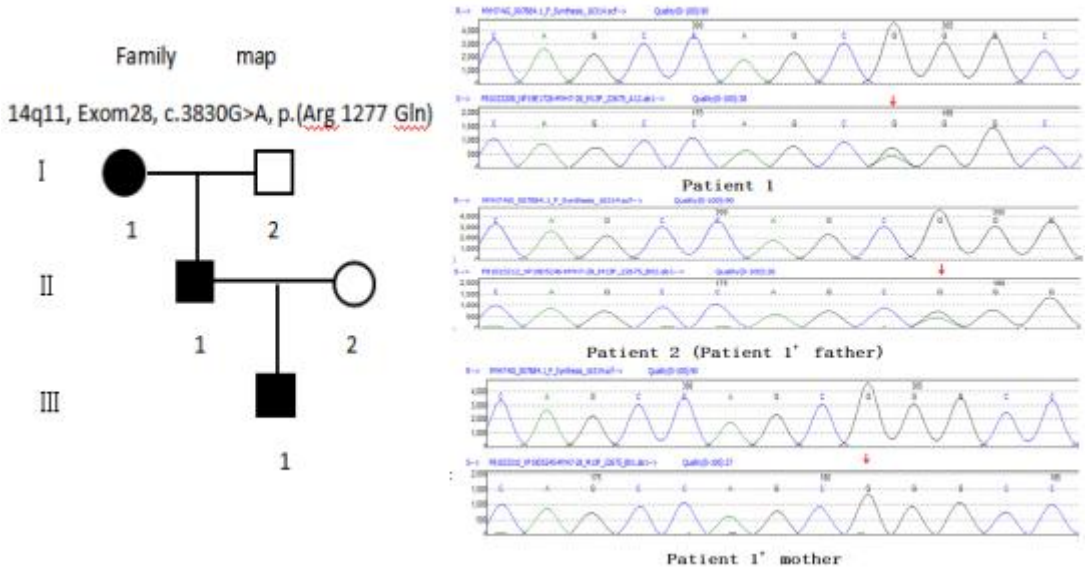

Figure 2

The genetic map and mutation sequence. Chromatograms in the MYH7 gene of this family. Black square: affected mutation-carrying male; White square: male without MYH7 mutation; White Circle: female without MYH7 mutation; Black circle: affected mutation-carrying female. The red arrows indicate the location of the identified mutation.