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Limb-Girdle Muscular Dystrophy R9 due to a Novel Complex Insertion/Duplication Variant in *FKRP* Gene

Erin Willis, MD[1], Steven A. Moore, MD, PhD[2], Mary O. Cox[2], Vikki Stefans, MD[3], Akilandeswari Aravindhan, MBBS[1], Murat Gokden, MD[4], and Aravindhan Veerapandiyan, MD[1]

Abstract

Limb-girdle muscular dystrophy R9 (LGMD2I, LGMDR9) is an autosomal recessive disorder caused by pathogenic variants in the fukutin-related protein (*FKRP*) gene. We describe a 17 year old boy with LGMDR9 whose symptoms began at age 5 years. Muscle histopathology, immunostaining, and western blotting were consistent with a dystroglycanopathy. Genetic testing identified maternal inheritance of the most common pathogenic *FKRP* variant c.826C>A (p.L276I). Also detected was a novel insertion and duplication on the paternally inherited *FKRP* allele: a single nucleotide insertion (c.948_949insC) and an eighteen nucleotide duplication (c.999_1017dup18) predicted to result in premature translation termination (p.E389*). Based on the clinical features and course of the patient, heterozygosity for the common pathogenic *FKRP* variant, and abnormal glycosylation of alpha-dystroglycan, we suggest that the novel *FKRP* insertion and duplication are pathogenic. This case expands the genetic heterogeneity of LGMDR9 and emphasize the importance of muscle biopsy for precise diagnosis.

Keywords

LGMD, LGMD 21, weakness, FKRP, dystroglycanopathy

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Introduction

Limb-girdle muscular dystrophy R9 (LGMD2I, LGMDR9) (OMIM: muscular dystrophy—dystroglycanopathy [limb-girdle], type C, 5) is an autosomal recessive disorder caused by pathogenic variants in the fukutin-related protein (*FKRP*) gene.[1] *FKRP*-associated muscular dystrophy is clinically heterogenous. The most severe phenotype, Walker-Warburg-like syndrome, is characterized by congenital hypotonia, progressive muscle weakness and atrophy, ocular and brain malformations, severe motor developmental delay, and profound intellectual disability. Milder phenotypes include congenital and limb-girdle muscular dystrophies with the onset of disease from infancy to adulthood and variable clinical course including asymptomatic elevated serum creatine kinase (CK), early onset rapidly progressive course with loss of ambulation in teenage years, late onset with slow progression, and primary cardiomyopathy with minimal skeletal muscle weakness.[1–5] LGMDR9 is typically characterized by proximal muscle weakness, calf hypertrophy, hypotonia, elevated CK level, normal cognition, and no structural brain abnormalities. Dilated cardiomyopathy and respiratory muscle weakness can be seen.[1]

[1]Division of Neurology, Department of Pediatrics, Arkansas Children's Hospital, University of Arkansas for Medical Sciences, Little Rock, AR, USA
[2]Department of Pathology, University of Iowa Carver College of Medicine and Senator Paul D. Wellstone Muscular Dystrophy Specialized Research Center, Iowa City, IA, USA
[3]Departments of Pediatrics and Physical Medicine and Rehabilitation, Arkansas Children's Hospital, University of Arkansas for Medical Sciences, Little Rock, AR, USA
[4]Department of Pathology, University of Arkansas for Medical Sciences, Little Rock, AR, USA

Corresponding Author:
Aravindhan Veerapandiyan, Arkansas Children's Hospital, University of Arkansas for Medical Sciences, 1 Children's Way, Slot 512-15, Little Rock, AR 72202, USA.
Email: aveerapandiyan@uams.edu

Pathogenic *FKRP* variants are the most prevalent cause of dystroglycanopathy. These variants reduce specific O-mannose-linked glycosylation of alpha-dystroglycan causing instability of the linkage between the dystrophin–glycoprotein complex and laminin alpha2 in the basement membranes of skeletal muscle fibers.[6] About 150 different pathogenic variants have been described in the *FKRP* gene to date. However, there is one common founder pathogenic variant that underpins most cases of LGMDR9 (c.826C>A; p.Leu276Ile). Patients who are homozygous for this variant typically have a milder phenotype, whereas compound heterozygotes have a relatively severe clinical course. In patients heterozygous for c.826C>A, clinical severity is very likely influenced by the variant in the second allele.[1,2] We describe a 17-year-old boy with LGMDR9 who has the c.826C>A mutation in *FKRP* on one allele and a novel complex insertion-duplication on the second allele. To the best of our knowledge, the complex insertion-duplication variant has not been reported to date.

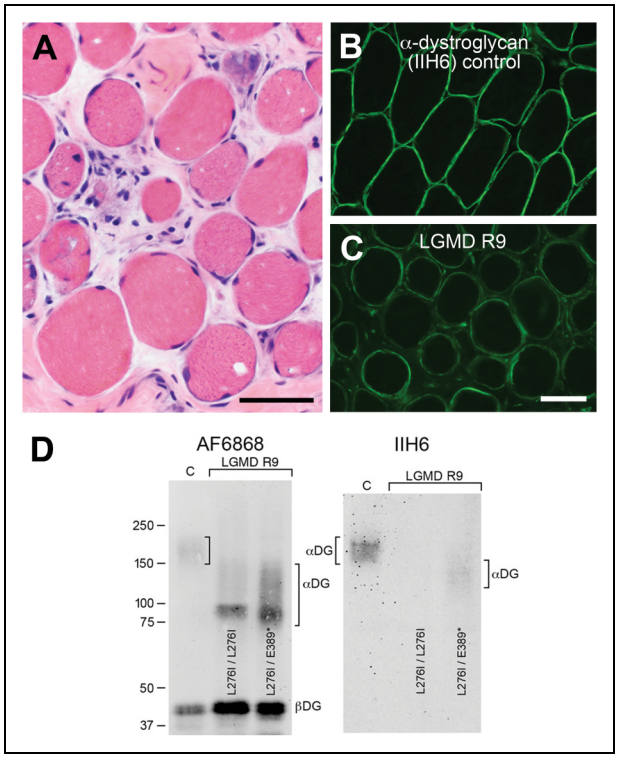

Figure 1. Muscle biopsy evaluation. (A) Frozen sections stained with H&E show the typical features of muscular dystrophy: fiber size variation due to atrophic and hypertrophic fibers, myonecrosis, regeneration, endomysial fibrosis and increased internal nuclei. (B, C). Immunofluorescence staining with the anti-alpha-dystroglycan antibody IIH6 shows bright circumferential staining of the sarcolemmal surface in control muscle (B) and a mosaic pattern of variably reduced staining in the patient's muscle (C). The size bars in panels A and C are 50 μm long. (D) Western blotting of wheat germ agglutinin (WGA) preparations of frozen muscle homogenates show reduced molecular weight alpha-dystroglycan (αDG) in the LGMDR9 samples (homozygous L276I and compound heterozygous L276I/E389*) when blotted with the AF6868 antibody that binds to amino acid epitopes in both αDG and βDG. Both LGMDR9 samples have reduced to absent binding to the glycosylation-dependent IIH6 antibody. C = normal control muscle; αDG = alpha-dystroglycan; βDG = beta-dystroglycan.

Case Report

Our patient is a now 17 year old boy who initially presented with weakness at the age of 5 years. He was born full term with no prenatal complications beyond mild gestational diabetes. Early developmental milestones were unremarkable except for a delay in independent walking until 19 months of age. At 5 years of age, he was noted to have difficulties with climbing stairs, running and hopping. Family history was unremarkable for any known neuromuscular disorders. Examination was significant for calf hypertrophy and lower extremity proximal greater than distal weakness (3/5 hip flexion, extension and abduction; 4/5 knee extension, knee flexion, ankle dorsiflexion and ankle plantarflexion bilaterally). He utilized a modified Gowers sign when transitioning from floor to standing and had decreased pelvic stability during gait with decreased speed and quality of movement while running. Laboratory workup at that time revealed elevated CK (7583 IU/L; normal range 4 to 88 IU/L). Magnetic resonance imaging (MRI) of the brain was normal. *DMD* gene deletion-duplication analysis and sequencing was unrevealing. He underwent vastus lateralis muscle biopsy with histopathology consistent with a dystrophic process (fiber size variation due to atrophic and hypertrophic fibers, endomysial fibrosis and increased internal nuclei; see Figure 1A) and immunostaining suggestive of a dystroglycanopathy [reduced staining for alpha-dystroglycan using a glycoepitope specific antibody (Figure 1C) with normal dystrophin, emerin, merosin, beta-dystroglycan and sarcoglycans staining]. Western blotting confirmed the hypoglycosylation of alpha-dystroglycan (Figure 1D).

FKRP gene sequencing was done at the age of 6 years. This showed a heterozygous pathogenic c.826C>A (p.L276I) variant in exon 4 of the patient's *FKRP* gene. The testing also detected two novel insertions on the other allele; a single nucleotide insertion (c.948_949insC) and an eighteen nucleotide duplication (c.999_1017dup18), resulting in a premature translation termination codon at position 389 (p.E389*). Parental testing demonstrated that the mother carried c.826C>A, while the father carried the insertion/duplication, confirming an autosomal recessive inheritance pattern. His parents are asymptomatic. Cardiology evaluation at the time of diagnosis showed no evidence of cardiac involvement. Pulmonary function tests were also unremarkable. Neuropsychological testing revealed frontal lobe and executive function deficit as well as attention deficit hyperactivity disorder. Given worsening of weakness, a prednisone trial was attempted when he was about 8 years old. He had progression of his weakness and became non-ambulatory by age 14 years. Prednisone was weaned at that point due to side effects including weight gain and worsening mood. The patient was closely monitored in our multidisciplinary neuromuscular clinic along with pulmonology and cardiology. He started using a mechanical insufflation –

exsufflation device twice daily at age 15 years. He underwent posterior spinal fusion with instrumentation for his scoliosis at the age of 16 years. At the most recent follow-up at the age of 17 years, he remained non-ambulatory using a motorized wheel chair for mobility. He was able to raise his hands to mouth but could not raise an 8 ounce glass of water to his mouth. Cardiac function remained stable.

Discussion

The patient described in this report has a clinical course consistent with LGMDR9. The first variant detected in our patient, Leu276Ile, is the most common *FKRP* pathogenic variant. This variant in homozygous form is associated with a milder phenotype and accounts for majority of patients with LGMDR9. Rare FKRP variants are increasingly recognized.[2,7] The insertion/duplication detected in our patient results in a transcript with a translational termination codon. This is predicted to result in a non-functional protein product even if the transcript escaped nonsense mediated decay. Based on the clinical features and course of the patient, compound heterozygosity with a common *FKRP* pathogenic variant in one allele, and reduced glycosylation of alpha-dystroglycan, we suggest that the novel insertion/duplication identified on the second allele is pathogenic, resulting in the LGMDR9 phenotype in our patient. It is speculated that this novel insertion/duplication variant in FKRP is responsible for a milder phenotype as seen in our patient.

We suggest that the single nucleotide insertion (c.948_949insC) and an eighteen nucleotide duplication (c.999_1017dup18) can be added to the repertoire of variants in *FKRP* associated with LGMDR9. Our report corroborates that the severity and clinical course of the LGMDR9 in compound heterozygous patients depend mainly on the second pathogenic variant. Moreover, this report highlights the importance of muscle biopsy and pathology in establishing a precise diagnosis when novel variants and/or variants of uncertain significance are detected by the genetic testing.

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Not applicable, because this article does not contain any studies with human or animal subjects.

Informed Consent

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ORCID iD

Aravindhan Veerapandiyan https://orcid.org/0000-0002-3065-3956

Trial Registration

Not applicable, because this article does not contain any clinical trials.

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