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

Erin Willis, MD1, Steven A. Moore, MD, PhD2, Mary O. Cox2, Vikki Stefans, MD3, Akilandeswari Aravindhan, MBBS1, Murat Gokden, MD4, and Aravindhan Veerapandiyan, MD1

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

Received January 28, 2022. Received revised April 6, 2022. Accepted for publication April 12, 2022.

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. FKRP-associated muscular dystrophy is clinically heterogeneous. 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. 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

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

**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 glycopeptide 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 –
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.

Acknowledgments

Dr Moore and Ms. Cox are supported by the Iowa Senator Paul D. Wellstone Muscular Dystrophy Specialized Research Center (MDSRC), National Institute of Neurological Disorders and Stroke of the National Institutes of Health under Award Number P50NS053672; Dr Moore is the Co-Director of this MDSRC. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Author Contributions

AV has received compensation for ad-hoc advisory boards/consulting activity with Biogen, Novartis, AveXis, Sarepta therapeutics, PTC therapeutics, Scholar Rock, Fibrogen, MDA, PPMD, and France Foundation outside of the submitted work. Other authors report no relevant disclosures.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Ethical Approval

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

Informed Consent

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

ORCID iD

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

Trial Registration

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

References

1. Amato AA. Other limb-girdle muscular dystrophies. Handb Clin Neurol. 2011;101:119-124. doi:10.1016/B978-0-08-045031-5.00008-6
2. Brown SC, Fernandez-Fuente M, Muntoni F, Vissing J. Phenotypic spectrum of alpha-dystroglycanopathies associated with the c.919T>a variant in the FKRP gene in humans and mice. J Neuropathol Exp Neurol. 2020;79(12):1257-1264. doi:10.1093/jn/enaa120
3. Mercuri E, Brockington M, Straub V, et al. Phenotypic spectrum associated with mutations in the fukutin-related protein gene. Ann Neurol. 2003;53(4):537-542. doi:10.1002/ana.10559
4. Schwartz M, Hertz JM, Sveen ML, Vissing J. LGMD2I Presenting with a characteristic Duchenne or Becker muscular dystrophy phenotype. Neurology. 2005;64(9):1635-1637. doi:10.1212/01.WNL.000157654.59374.ES
5. Sveen ML, Schwartz M, Vissing J. High prevalence and phenotype-genotype correlations of limb girdle muscular dystrophy type 2I in Denmark. Ann Neurol. 2006;59(5):808-815. doi:10.1002/ana.20824
6. Yoshida-Moriguchi T, Campbell KP. Matriglycan: a novel polysaccharide that links dystroglycan to the basement membrane. Glycobiology. 2015;25(7):702-713. doi:10.1093/glycob/cwv021
7. Saylam E, Moore SA, Aravindhan A, et al. A novel noncoding FKRP mutation in early onset limb-girdle muscular dystrophy. Neurol Genet. 2020;6(1):e388. doi:10.1212/NXG.0000000000000388