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A novel noncoding *FKRP* mutation in early onset limb-girdle muscular dystrophy

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Limb-girdle muscular dystrophy 2I (LGMD2I, LGMD R9) (OMIM: muscular dystrophy—dystroglycanopathy [limb-girdle], type C, 5) is an autosomal recessive disorder caused by mutations in the fukutin-related protein (*FKRP*) gene.[1] The phenotypic spectrum associated with *FKRP* is 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 the onset of disease from infancy to adulthood and variable clinical course including asymptomatic elevated serum creatinine 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.[1,2] LGMD2I 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] Mutations in the *FKRP* gene reduce specific O-mannose-linked glycosylation of alpha-dystroglycan, leading to the instability of the linkage between the dystrophin–glycoprotein complex and laminin alpha 2 in the basement membrane of skeletal muscle.[3] The most common *FKRP* mutation seen in LGMD2I is c.826C>A (p.Leu276Ile). Patients who are homozygous for this mutation typically have a milder phenotype, whereas compound heterozygotes have a relatively severe clinical course. In patients heterozygous for c.826C>A, clinical severity may correspond best to the mutation in the second allele.[1,4] We describe a 9-year-old boy with LGMD2I who has the c.826C>A mutation in *FKRP* on 1 allele and a novel variant on the second allele.

Our patient presented with a 3-year history of slowly progressive muscle weakness. He was born at term after a normal pregnancy. His growth and early developmental milestones were normal. Initial symptoms included leg pain[5] and toe walking that was noticed around 6 years of age. Over the next 3 years, he exhibited difficulties with running, jumping, rising from the floor, and climbing stairs. His medical, family, and neurodevelopmental history was otherwise unremarkable. Neurologic examination revealed proximal limb-girdle weakness, truncal weakness, hypotonia, positive Gowers sign, and waddling gait. Musculoskeletal examination showed calf hypertrophy and tight heel cords. The remainder of the examination was normal. Serum CK level was 9701 U/L. EMG of the lower extremity muscles suggested a myopathic process. The initial next generation sequencing (Invitae comprehensive muscular dystrophy panel of 48 genes) revealed the known pathogenic variant, c.826C>A (p.Leu276Ile), in *FKRP* exon 4. This panel covered coding exons and only ± 10 base pairs of adjacent intronic sequence of each gene, and variants outside these would not be detected. Subsequently, trio whole exome sequencing confirmed the presence of this variant inherited from his father and identified a novel variant in *FKRP*, c.-253+4A>G (Chr19:47249351 [GRCh37] ENST00000391909.7) in intron 1, inherited from his mother. No variants were identified in the other genes associated with dystroglycanopathies.[6] His parents are asymptomatic. He

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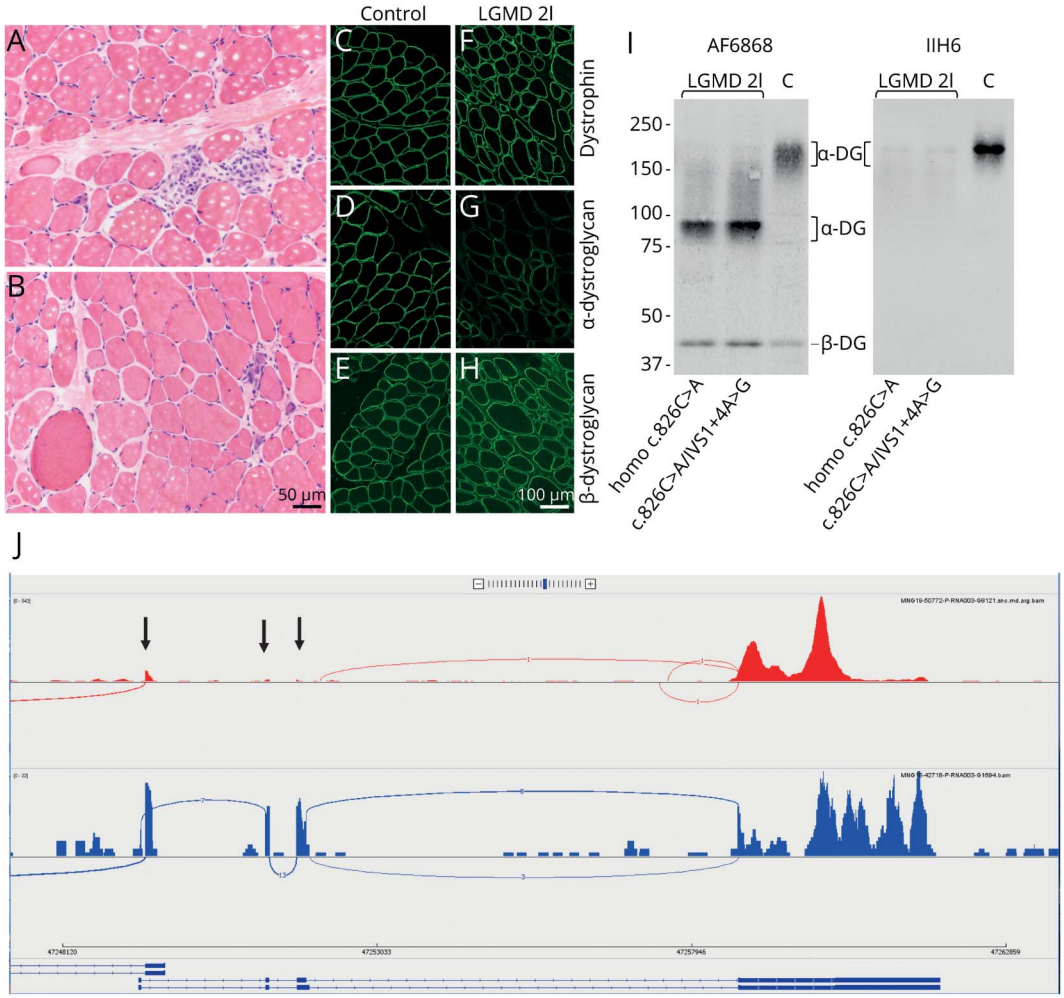
Figure Muscle biopsy (A–I): many histopathologic changes characteristic of a muscular dystrophy are present in the patient's quadriceps biopsy: myonecrosis and regeneration, atrophy and hypertrophy, and endomysial fibrosis (A and B)



Immunofluorescence evaluation of dystrophin, alpha-dystroglycan, and beta-dystroglycan were performed as described.[7] There is selectively reduced staining for alpha-dystroglycan using a matriglycan-specific antibody, IIH6 (C–H). The mosaic pattern of reduced to absent immunostaining is characteristic of milder dystroglycanopathy phenotypes (G). Western blotting of pooled muscle biopsy cryosections followed the methods described previously.[7] Blotting with a core peptide antibody, AF6868, shows that our patient has reduced molecular weight alpha-dystroglycan that is very similar to a patient with homozygous c.826C>A *FKRP* mutations. Normal control muscle is designated as "C". Almost no fully glycosylated alpha-dystroglycan is detected by IIH6 in either our patient or the patient with homozygous c.826C>A *FKRP* mutations (I). RNA sequencing (J): Sashimi plot of *FKRP* expression in muscle of our patient (red) and control (blue). Expression of the 3 non-coding exons of *FKRP* is absent in the patient compared with control (arrows). RNA sequencing examination revealed 0–12 reads covering the untranslated exons of the *FKRP* gene and 31–543 reads covering the translated exons in our patient. In comparison, 2–15 reads covering the untranslated exons of the *FKRP* gene and a similar number of reads covering the translated exons (0–22 reads) were seen in control sample. Junction reads spanning the non-coding exons are present in the control sample and are essentially absent (≤1) in the patient. This indicates a disruption in the splicing between exons 1, 2, 3, and 4 in the patient which is consistent with the c.-253+4A>G variant affecting normal splicing. FKRP = fukutin-related protein; LGMD = limb-girdle muscular dystrophy.

then underwent muscle biopsy that showed a dystrophic process: prominent fiber size variation, myonecrosis, regeneration, and fibers with increased internal nuclei sometimes associated with fiber splitting. No neurogenic or inflammatory changes were noted. Immunostaining revealed a mosaic pattern of reduced to absent staining for alpha-dystroglycan using the matriglycan-specific antibody IIH6 (figure). Immunostaining for dystrophin, beta-dystroglycan, and spectrin were normal. Western blotting confirmed the glycosylation abnormality by showing reduced molecular weight of alpha-dystroglycan and near absence of binding to IIH6. Echocardiogram was normal.

The Leu276Ile variant is the most common *FKRP* pathogenic variant.[1] The second variant found in our patient, c.-253+4A>G, has not been reported previously. Because this variant destroys the canonical splice donor site in intron 1, it is expected to cause abnormal gene splicing. Several *in silico* analyses (MutationTaster, NNSplice, and NetGene2) predict this variant is likely to affect splicing. Indeed, RNA sequencing in our patient confirmed that the c.-253+4A>G variant disrupts normal splicing (figure). Thus, based on the clinical features, compound heterozygosity with a common *FKRP* mutation in 1 allele, reduced immunostaining for alpha-dystroglycan, abnormal glycosylation by western blot,

and abnormal splicing caused by the second variant, we speculate that the novel *FKRP* variant in second allele is associated with the LGMD2I phenotype in our patient.

We suggest that the variant c.-253+4A>G can be added to the repertoire of variants in *FKRP* associated with LGMD2I. Whole exome sequencing may be needed in patients with strong clinical suspicion for etiologic delineation in the event of ambiguous results on widely available next generation sequencing panel testing. Furthermore, this report emphasizes the importance of muscle biopsy for establishing a precise diagnosis when genetic testing results are inconclusive.

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