Unexpected partial RNA deletion by two different novel COL6A2 mutations leads to Ullrich congenital muscular dystrophy

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Abstract

Limb weakness is an uncommon symptom in children, with multiple factors contributing to related diseases, particularly genetic disorders. A nine-year-old boy presented with slowly progressive muscle weakness of the limb-girdle muscles. We evaluated the clinical symptoms, laboratory tests, imaging examinations, and pathological examinations of this proband. We combined whole-exome and Sanger sequencing to identify the novel compound heterozygous pathogenic mutations NM 001849.3: c.1970-10_1978 del CGGCTTGCAGGGACGCGTG and c.2462-3C>A in *COL6A2* in this proband inherited from the mother and father, respectively. Mutational confirmation at the mRNA level demonstrated that the proband carried a homozygous abnormal sequence with 23bp deletions (c.2462-2484 del GGACGCGTGTGGGCGTGGTGCAG) at the beginning of exon 26. In contrast, both parents and sibling have normal sequences with no clinical symptoms. The results of this study further expand the mutational spectrum and will be helpful for further molecular diagnosis.

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Keywords: COL6A2; Ullrich congenital muscular dystrophy (UCMD); compound heterozygous mutations; whole exome sequencing; sanger sequencing;

Congenital muscular dystrophies (CMDs) are a group of rare genetic diseases that primarily affect the muscle and are characterized by progressive degeneration and weakness(Kirschner, 2013). Clinical symptoms typically manifest at birth or within the first few months of life(Carakushansky et al., 2012). Ullrich congenital muscular dystrophy (UCMD) is a rare type of autosomal dominant or recessive CMDs, mainly caused by mutations in the related genes leading to loss of collagen VI with an earlier onset time and progressive clinical symptoms(Kirschner, 2013; Park et al., 2014). Collagen type VI is consistently linked to the development of congenital muscular dystrophy(Bushby et al., 2014; Antoniel et al., 2020; Simsek-Kiper et al., 2020), composed of three distinct alpha chains encoded by *COL6A* (Lampe & Bushby, 2005). Most patients with UCMD carry mutations in *COL6A* that result in the abnormal production of collagen type VI(Bushby et al., 2014). However, the relationship between genotype and phenotype remains unclear.

The nine-year-old boy **(II-2, Figure 1 A**) was the second child of healthy non-consanguineous parents. The patient presented myasthenia and was initially diagnosed with CMD. His symptoms of gait instability had not improved and became increasingly severe as the proband got older; intellectual development was normal compared with peers. Physical examination shows a clear mind, mental reactivity, normal nutritional condition, bilateral lower extremity weakness, the inability to stand, and the left positive Babinski sign. The proband presented equinus when he stood with auxiliary assistance. The muscle strength of the upper limbs was grade IV, and that of the lower limbs was III. Deep tendon reflexes were decreased in the knees. Auxiliary examinations yield the following results: Laboratory examinations revealed no obvious abnormalities in the blood routine or coagulation parameters. Biochemical assays were as follows: creatine kinase isoenzyme MB 12.03 (Reference value: 0–5 ng/ml); creatinine 19 (Reference value: 62–115 μmol/L); creatine kinase 370 (Reference value: 38–174 U/L). On the biceps brachii, vastus medialis, and anterior muscle, needle electromyography revealed that the duration of voluntary motor unit potential on volition was reduced by 32%, 27%, and 38%, respectively. There were no discernible abnormalities in the upper and lower limb sensory nerve conduction. Similarly, no abnormalities were detected during brain MRI or diffusion-weighted imaging (DWI). The left biceps brachii muscle biopsy was performed **Figure 1 B** . The pathological outcome was the atrophy of both types of fibers. Part of the muscle fiber was replaced by connective and adipose tissues, and the size of the remaining muscle fiber was noticeably different. A small amount of regenerative and moth-eaten muscle fiber also appeared. Immunohistochemistry results indicated that dystrophin-Rod, dystrophin-N, and β-sarcoglycan were reduced in muscle fiber membranes compared to the normal control. A proportion of muscle fiber membranes were absent of dystrophin-C reporter staining. Collectively, the proband is highly probable to have muscular dystrophy, and genetic testing is extremely helpful for disease diagnosis.

Our whole-exome sequencing (WES) results indicate that the proband carries two novel compound heterozygous pathogenic mutations in*COL6A2* , one of which was a frameshift mutation (NM 001849.3: c.1970-17_-1971 del ACGCGTGCGGCTTGCAGGG), and the other was a splice site mutation (NM 001849.3: c.2462-3C>A) (**Figure 1 C**). Subsequently, the mutations of *COL6A2* in the proband, his parents, and his sibling were validated by Sanger sequencing. Notably, the frameshift mutation (c.1970-10_1978 del CG-GCTTGCAGGGACGCGTG) was confirmed at the DNA level in the proband and his mother by Sanger sequencing and was different from the WES results Moreover, another site was observed in the father and proband. The mode of inheritance complies with the rules of autosomal dominant inheritance. The mRNA was extracted from the peripheral blood to further verify the functional effect of the genetic variants. RNA sequence analyses showed wild-type sequences in the parents and sibling. However, the proband has a homozygous but unusual sequence, a 23bp (c.2462–2484 del GGACGCGTGTGGGCGTGGTGCAG) deletion

at the beginning of exon 26 compared to wild-type (**Figure 1 D**). We speculated that mutations affect the phenotype only when two mutations are present together.

Zhang et al. reported two UCMD patients with different mutations in*COL6A2* ; one carried a homozygous c.1870G>A (p.E624K) variant and the other a homozygous c.2626C>A (p.R876S). Both patients presented with myasthenia, joint contractures, and joint laxity(Zhang et al., 2010). Lucarini et al. reported a proband who carried a homozygous A > G mutation at -10 of intron 12 in*COL6A2* that goes along with generalized muscle weakness, arthrogryposis, and mild spine rigidity(Lucarini et al., 2005). In our study, we described a UCMD proband with slowly progressive muscle weakness of the limb-girdle muscles and identified novel compound heterozygous pathogenic mutations of *COL6A2* in a Chinese family. Subsequently, we directly extracted RNA from peripheral blood to verify the effect of the two different novel types of *COL6A2* mutations at the mRNA level. Our results demonstrated that the proband carried a homozygous aberrant mRNA sequence, suggesting that two different novel mutations may together contribute to the partial deletion at the beginning sequences of the same exon 26. This might be attributed to the defective pre-mRNA splicing and phenotype caused only when both mutations are present, indicating that RNA sequencing is also essential to diagnose the disease. However, the action mechanism of those mutations is not entirely clear. Therefore, an ideal method combining DNA and RNA sequencing has been suggested as an optimal tool for prenatal diagnosis. In summary, our research demonstrated the novel pathogenicity of mutations and expanded the variant spectrum of *COL6A2* , further improving the accurate diagnosis of UCMD.

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Conflict Of Interest Statement

The authors declared that they have no conflicts of interest to this work.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.**Disclosure**The authors report no disclosures.

References

Antoniel, M., Traina, F., Merlini, L., Andrenacci, D., Tigani, D., Santi, S., Cenni, V., Sabatelli, P., Faldini, C., & Squarzoni, S. (2020). *Tendon Extracellular Matrix Remodeling and Collagen VI Mutations* .

Bushby, K. M. D., Collins, J., & Hicks, D. (2014). Collagen type VI myopathies. *Advances in Experimental Medicine and Biology* ,*802* , 185–199. https://doi.org/10.1007/978-94-7-7893-1_12

Carakushansky, G., Ribeiro, M. G., & Kahn, E. (2012). Moderately progressive Ullrich congenital muscular dystrophy. *Jornal de Pediatria* , *88* (1), 93–96. https://doi.org/10.2223/JPED.2112

Kirschner, J. (2013). Congenital muscular dystrophies. In *Handbook of Clinical Neurology* (1st ed., Vol. 113). Elsevier B.V. https://doi.org/10.1016/B978-0-444-59565-2.00008-3

Lampe, A. K., & Bushby, K. M. D. (2005). Collagen VI related muscle disorders. *Journal of Medical Genetics* , *42* (9), 673–685. https://doi.org/10.1136/jmg.2002.002311

Lucarini, L., Giusti, B., Zhang, R. Z., Pan, T. C., Jimenez-Mallebrera, C., Mercuri, E., Muntoni, F., Pepe, G., & Chu, M. L. (2005). A homozygous COL6A2 intron mutation causes in-frame triple-helical deletion and nonsense-mediated mRNA decay in a patient with Ullrich congenital muscular dystrophy. *Human Genetics* , *117* (5), 460–466. https://doi.org/10.1007/s00439-005-1318-8

Park, Y., Park, M. S., Sung, D. H., Sohn, J. Y., Ki, C. S., & Kim, D. H. (2014). Ullrich congenital muscular dystrophy possibly related with COL6A1 p.Gly302Arg variant. *Annals of Rehabilitation Medicine* ,*38* (2), 292–296. https://doi.org/10.5535/arm.2014.38.2.292

Simsek-Kiper, P. O., Oguz, S., Ergen, F. B., Utine, G. E., Alikasifoglu, M., & Haliloglu, G. (2020). A Revisited Diagnosis of Collagen VI Related Muscular Dystrophy in a Patient with a Novel COL6A2 Variant and 21q223 Deletion. *Neuropediatrics* , *51* (6), 445–449. https://doi.org/10.1055/s-0040-1714125

Zhang, R. Z., Zou, Y., Pan, T. C., Markova, D., Fertala, A., Hu, Y., Squarzoni, S., Reed, U. C., Marie, S. K. N., Bönnemann, C. G., & Chu, M. L. (2010). Recessive COL6A2 C-globular missense mutations in Ullrich congenital muscular dystrophy: Role of the C2a splice variant.*Journal of Biological Chemistry* , *285* (13), 10005–10015. https://doi.org/10.1074/jbc.M109.093666

Fig. 1. Genetic and pathological findings of the proband and his parents.

(A) Pedigree of the family with UCMD. The black arrow denotes the proband. B and C indicate the partial DNA sequence chromatograms of splice site mutation in COL6A2. (B) The frameshift mutation (c.1970-10_1978delCGGCTTGCAGGGACGCGTG) in the proband and his mother. (C) The splice site mutation (c.2462-3C>A) in the proband and his father. The black arrows denote the mutation site. (D) Partial RNA sequence chromatograms of the frameshift mutation in COL6A2 show a 23 bp-deletion (GGACG-CGTGTGGGCGTGGTGCAG) compared to wild-type. The black arrow indicates the start of the deletion site. (E) Hematoxylin and eosin staining results demonstrate that part of the muscle fiber is replaced with connective and adipose tissues; the sizes are significantly different in the remaining myofibers. Fragmenting and hypercontracted myofibers are visible. The number of fibers with internal nuclei and nuclear bags were evidently increased. (F) The immunohistochemical results indicate that the staining of myofiber membranes is diffusely weakened and even absent (100 μm).