Case Report

Identification of *LAMA2* compound heterozygous variants: a case report

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Background: Laminin-α2 (*LAMA2*) chain-deficient muscular dystrophy (*LAMA2*-MD) is the most common congenital muscular dystrophy (CMD) in the world. Its main manifestations are muscle weakness and hypotonia that occur after birth or at early infancy.

Case Description: We reported a case of a 3-year-old and 6-month-old boy presented with delayed motor development, elevated creatine kinase (CK) levels, and abnormal white matter in the brain. Whole exome sequencing (WES) showed compound heterozygous variants of the *LAMA2* gene. This case reports for the first time the compound heterozygous *LAMA2* variants c.5476C>T (p.R1826*) (paternal inheritance) with c.2749 + 2dup (maternal inheritance), as both variants are interpreted as pathogenic/potentially pathogenic variants.

Conclusions: This study reports a novel heterozygous variant, including two pathogenic variants in the *LAMA2* gene, and highlights the effectiveness of highly efficient exome sequencing applying in patients with undefined CMDs.

Keywords: Laminin-α2 (*LAMA2*); merosin-deficient congenital muscular dystrophy (merosin-deficient CMD); limb-girdle muscular dystrophy; whole exome sequencing (WES); case report

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Introduction

Congenital muscular dystrophies (CMDs) are phenotypic heterogeneous diseases. Its main manifestations are muscle weakness and hypotonia that occur after birth or at early infancy (1). CMDs are not common, with an incidence of 0.82/100,000 in live births (1). According to previous reports, 37 genes have been identified to be associated with CMDs (2,3). Laminin-α2 (*LAMA2*) chain-deficient muscular dystrophy (*LAMA2*-MD) is the most common CMD in the world, which affects about 36.4% in China (4).

LAMA2-MD is an autosomal recessive genetic disorder caused by variants in the *LAMA2* gene leading to the decreased or absent production of protein LAMA2, which leads to a deficiency of laminin-211 and/or laminin-221, resulting in a decrease in the strength and stability of skeletal muscle. Based on the degree of LAMA2 deficiency,

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LAMA2-MD varies from severe merosin-deficient CMD type 1A (MDC1A, OMIM # 607855) to milder late-onset limb-girdle MD-23 (LGMDR23, OMIM # 618138). Blood biochemical indicators such as elevated serum creatine kinase (CK) levels, immunohistochemistry results mainly from skin or muscle biopsies, and brain magnetic resonance imaging (MRI) findings such as white matter abnormalities can be used to diagnose MDC1A (5,6). It is currently recommended to use next-generation sequencing (NGS) for genetic testing in *LAMA2*-MD (7,8).

There are over 800 unique variants that have been reported in the *LAMA2*. Among them, a total of 666 pathogenic variants in the *LAMA2* gene have been reported in the Human Gene Mutation Database (HGMD) Professional release 2023.4 (http://www.hgmd.cf.ac.uk/ac/gene.php?gene=LAMA2) (*Table 1*). Furthermore, according to the Leiden Open Variation Database (LOVD, v3.0), 2.64% cases with more than two variants and 30.53% individuals with two variants in the *LAMA2* gene.

Here, we report a boy presenting a delayed motor development, and elevated CK levels, with compound heterozygous variants of the *LAMA2* gene. We present this case in accordance with the CARE reporting checklist (available at https://tp.amegroups.com/article/view/10.21037/tp-24-62/rc).

Highlight box

Key findings

- Through exome sequencing, this study reports a novel heterozygous variant in the laminin-α2 (*LAMA2*) gene in a boy manifesting as congenital muscular dystrophy (CMD).

What is known and what is new?

- *LAMA2* chain-deficient muscular dystrophy is an autosomal recessive genetic disorder caused by variants in the *LAMA2* gene leading to the decreased or absent production of protein LAMA2.
- This case reports for the first time the compound heterozygous *LAMA2* variants c.5476C>T (p.R1826*) (paternal inheritance) with c.2749 + 2dup (maternal inheritance), as both variants are interpreted as pathogenic/likely pathogenic variants.

What is the implication, and what should change now?

- Whole exome sequencing is an effective method for diagnosing CMD, a helpful tool for genetic counseling and prenatal testing. In the future, it is necessary to evaluate the impact of identified variants on *LAMA2* function through experiments to promote understanding of the CMD pathogenesis and personalized gene therapy for patients.

Table 1 Pathogenic mutations in the *LAMA2* gene in HGMD

Mutation type	Number of mutations	Proportion (%)
Missense/nonsense	310	46.55
Splicing	106	15.92
Regulatory	0	0
Small deletions	131	19.67
Small insertions	49	7.36
Small indels	1	0.15
Gross deletions	50	7.51
Gross insertions/duplications	15	2.25
Complex rearrangements	4	0.60
Repeat variations	0	0
HGMD Professional 2023.4 total	666	100

LAMA2, laminin-α2; HGMD, Human Gene Mutation Database.

Case presentation

The proband was a boy aged 3 years and 6 months who had been seeking medical attention for high CK levels. This boy can only walk when he was 15 months old. At the age of 3, there was a noticeable lack of running, limb weakness, inability to perform leg jumps, and poor stair walking, with good language development. There was no history of birth rescue, allergies, nor major illnesses. Screening for major genetic metabolic diseases in newborns was shown negative. He was the first and only child of the family and there was no family history of hereditary diseases. No obvious positive signs were found in the physical examination. Neurological examination showed cranial nerve negative, limb muscle strength grade IV, hypotonia, normal tendon reflex, abdominal wall reflex led out, negative Babinski sign, and mild hypertrophy of gastrocnemius muscle. Laboratory tests found that his serum CK level was as high as 987 U/L. Thyroid function test showed no abnormalities. TORCH test (panel for four serious infectious conditions in infants: toxoplasmosis, rubella, cytomegalovirus and herpes simplex virus) showed an increase of 105 U/mL (normal range, <14 U/mL) in cytomegalovirus antibody immunoglobulin G (IgG). The electromyogram (EMG) showed increased multiphase waves and shortened duration of motor units of limb muscles, indicating myogenic lesions. Brain MRI results showed symmetrical abnormal signals in bilateral

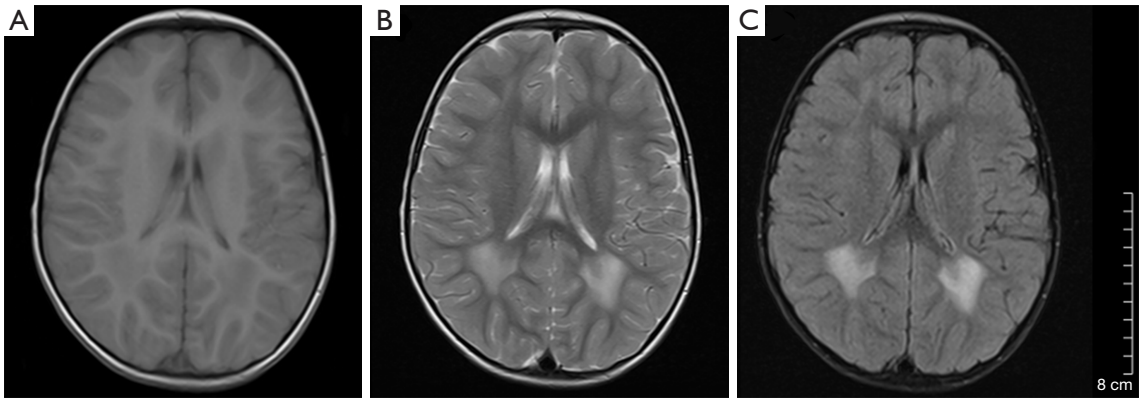

Figure 1 Brain MRI. Abnormal signal symmetry in the deep white matter of both sides of the brain with slight hypointensity on T1 (A), slight hyperintensity on T2 (B) and FLAIR (C). MRI, magnetic resonance imaging; FLAIR, fluid attenuated inversion recovery.

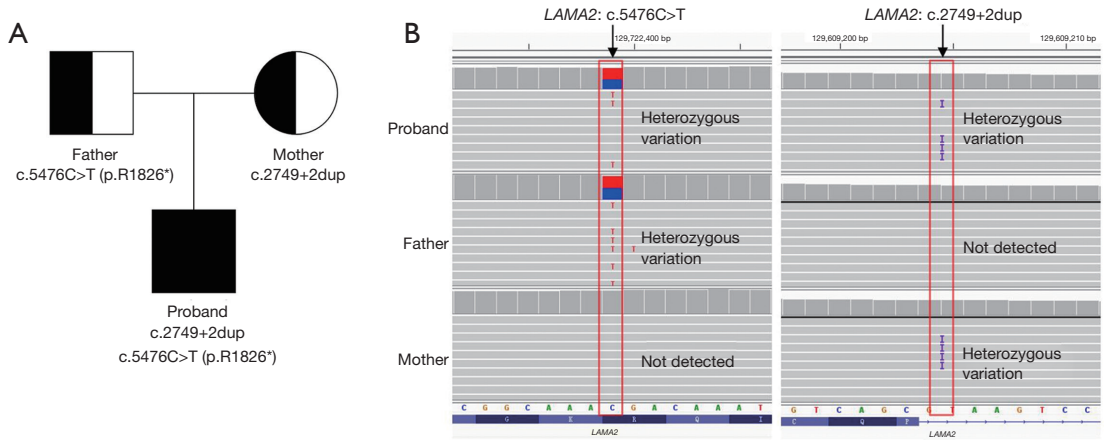

Figure 2 Information of *LAMA2* mutation in the family of the proband. (A) Family map of gene mutation. (B) Next-generation sequencing of mutation. *LAMA2*, laminin-α2.

deep white matter of the brain (*Figure 1*). Patients with white matter abnormalities may have a risk of epilepsy (9,10), but no prominent epileptiform activity was observed in the electroencephalogram (EEG) of this patient. No specific changes were found in the genetic metabolism map. A trio-based whole exome sequencing (WES) on genomic DNA was performed (Department of Screening Laboratory). The results showed compound heterozygous *LAMA2* variants, c.5476C>T (p.R1826*) that was paternally inherited, and c.2749 + 2dup that was maternally inherited (*Figure 2*). Both variants were interpreted as pathogenic/likely pathogenic variants, suggesting a diagnosis of *LAMA2*-MD.

This study was reviewed and approved by the Ethics Committee of The Children's Hospital Zhejiang University School of Medicine (Ref. 2024-IRB-0038). All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the participant's legal guardian for the publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office of this journal.

Discussion

In addition to the delayed motor development of this case, other main diagnostic evidences were: elevated CK level, EMG-suggested myogenic lesions, and brain MRI-shown symmetrical abnormal signals in bilateral deep white

matter. Importantly, the proband of this case inherited chromosomes from parents who were with different pathogenic variants in *LAMA2* gene: c.5476C>T(p.R1826*) and c.2749 + 2dup (*Figure 2*), conforming the diagnosis of *LAMA2*-MD.

This is a relatively mild case of MDC1A, with the following phenotypic characteristics: (I) delayed diagnose until he came to the department of neurology at 3 years and 6 months old. He received routine follow-up at the local health care clinic and was not promptly referred to a higher-level hospital, despite suffering from early onset of hypotonia and inability to walk and exhibiting an obvious delayed motor development at the age of 3. (II) CK level is relatively low, as in severe cases of MDC1A, the serum CK level is usually between several thousand to ten thousand. This relatively low CK level indicates that the muscle damage is not very serious, so the symptoms displayed are also mild, which can easily lead to negligence. (III) No CMD-family history and neither parent shows the phenotype.

For genotype characteristics, the compound variants occurred in this case with them originating from the parents separately. In exon 38 of the *LAMA2* gene, a nonsense variant c.5476C>T(p.R1826*) is inherited from the father. The identified rare *LAMA2* c.5476C>T was pathogenic, classified as PSV1, PM3, PP3, and PP5 according to the American College of Medical Genetics and Genomics (ACMG) guidelines classification (11). And the variant is expected to lose function due to premature protein truncation or nonsense-mediated mRNA decay. Based on data from the Genome Aggregation Database (gnomAD), the *LAMA2* c.5476C>T variant is observed in <0.0001 (7/251,122) of total alleles studied, and the frequency of the East Asian subgroup is 0.0001 (2/18,386). This variant was mainly detected in the compound heterozygous state (12-14). The c.2749 + 2dup is a splicing variant inherited from the mother and has been reported in previous studies (15,16). The splice donor site duplication (c.2749 + 2dup) may lead to splicing abnormalities. The identified rare *LAMA2* c.2749 + 2dup was likely pathogenic classified as PM3, PP1, PP3, PP4 and PP5 according to the ACMG guidelines classification (11). GnomAD shows that the overall frequency of this variant in the population is 1/250,932 (with a frequency of 0/18,390 in the East Asian population), and it is not supported as a polymorphic locus. Results from a study by using spliceAI analysis suggest this variant may affect splicing (15), but it has not been experimentally investigated (17). Although this variant achieved a high spliceAI score of 0.99 for splice altering (15), it is also possible that the in frame/out of frame exon skips or the splice donor site changes to another location could lead to mild phenotypes in patients like this one (17). This variant is not related to complete loss-of-function of the gene (16) and is consistent with the observed in our case. Besides, a previous study reported that c.2749 + 2dup might be related to white matter abnormalities (16), as observed in this case of brain MRI. Due to the expression of *LAMA2* in cerebral blood vessels, it may be important for the selective filtering ability of the blood-brain barrier. Dysfunction of *LAMA2* may lead to impaired selective filtering, resulting in plasma component leakage and central nervous system damage (18).

In addition, according to ClinVar Database, there are currently five reported cases of *LAMA2*: c.5476C>T, and two reported cases of *LAMA2*: c.2749 + 2dup (*Table 2*) (12-16). Currently (by January 2024), the *LAMA2*-LOVD (v3.0), an open-source DNA variation database system, contains a total of 2,628 public reported *LAMA2*-related variants (871 unique) identified in a total of 1,703 individuals (https://databases.lovd.nl/shared/genes/LAMA2). There were 45 cases with more than two variants, and 520 individuals with two variants. This case reports for the first time the compound heterozygous *LAMA2* variants c.5476C>T (p.R1826*) (paternal inheritance) with c.2749 + 2dup (maternal inheritance), as both variants are interpreted as pathogenic/likely pathogenic variants.

Furthermore, diagnosis requires muscle biopsy, immunohistochemical staining, or genetic testing. It was reported that there was no merosin staining in MDC1A, but low levels of residual merosin can be detected in LGMDR23 (15,19). Unfortunately, muscle biopsy and immunohistochemistry staining had not been performed in this case. Thyroid function test is to exclude hypothyroid myopathy. TORCH testing is to exclude CK elevation caused by infectious myositis. The positive cytomegalovirus IgG antibody indicated that the patient had previously been infected with cytomegalovirus and not related to the current increase in CK.

Altogether, WES is an effective method for diagnosing CMD, a helpful tool for genetic counseling and prenatal testing. In the future, it is necessary to evaluate the impact of identified variants on *LAMA2* function through experiments to promote understanding of the CMD pathogenesis and personalized gene therapy for patients.

Table 2 Reported cases of the same variants in the *LAMA2* gene with our case

Patient	CK	EMG	Muscle biopsy	MRI	NGS	Inheritance
P1 (13)	N/A	N/A	+	N/A	c.8038delG	N/A
					c.5476C>T	N/A
P2 (14)	+	+	+	+	c.2958G>A	Paternal
					c.5476C>T	Maternal
P3 (14)	+	−	+	+	c.7987delG	Paternal
					c.5476C>T	Maternal
P4/P5 (12)	+	+	+	+	c.5525C>T	Paternal
					c.43511-1G>C	Maternal
P6 (15)	+	+	N/A	+	c.2749 + 2dup	N/A
					c.8689C>T	N/A
P7 (16)	+	N/A	N/A	+	c.2749 + 2dup	Paternal
					c.1338_1339del	Maternal
P8 (our case)	+	+	N/A	+	c.5476C>T	Paternal
					c.2749 + 2dup	Maternal

LAMA2, laminin-α2; CK, creatine kinase; EMG, electromyogram; MRI, magnetic resonance imaging; NGS, next-generation sequencing; N/A, not available.

Conclusions

The dual pathogenic variants in the *LAMA2* gene produce a series of CMDs (*LAMA2*-MDs). By using WES, we identified two variants in the *LAMA2* gene in a boy. The variant c.5476C>T (p.R1826*) is expected to lose function due to premature protein truncation or nonsense-mediated mRNA decay and is classified as pathogenic. The splicing variant c.2749 + 2dup is also interpreted as pathogenic variants. The predominant clinical manifestations observed in this patient included delayed motor development, elevated CK levels, and abnormal white matter in the brain. This report emphasizes the effectiveness of WES application in undefined CMD patients.

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Footnote

Reporting Checklist: The authors have completed the CARE reporting checklist. Available at https://tp.amegroups.com/article/view/10.21037/tp-24-62/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tp.amegroups.com/article/view/10.21037/tp-24-62/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was reviewed and approved by the Ethics Committee of The Children's Hospital Zhejiang University School of Medicine (Ref. 2024-IRB-0038). All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee(s) and

with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the participant's legal guardian for the publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office of this journal.

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