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A novel *de novo* variant of *LAMA2* contributes to merosin deficient congenital muscular dystrophy type 1A: Case report

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Abstract. Merosin deficient congenital muscular dystrophy type 1A (MDC1A) is caused by defects in the *LAMA2* gene. Patients with MDC1A exhibit severe symptoms, including congenital hypotonia, delayed motor development and contractures. The present case report describes a Vietnamese male child with clinical manifestations of delayed motor development, limb-girdle muscular dystrophy, severe scoliosis and white matter abnormality in the brain. Whole exome sequencing (WES) was performed with subsequent validation using Sanger sequencing, and a *de novo* missense variant (NM_000426.3:c.1964T>C, p.Leu655Pro) and a splice site variant (NG_008678.1:c.3556-13T>A) in the *LAMA2* gene of the proband was detected. The missense variant located in exon 14 and has not been reported previously, to the best of our knowledge; whereas the splice site variant has been previously reported to cause premature termination of transcription in patients with MDC1A. In silico tools predicted that the missense variant was damaging. Phenotype-genotype analysis suggested that this proband was associated with classical early onset MDC1A. The co-existence of a *de novo* and a heterozygous variant in the *LAMA2* gene suggested that the *de novo* variant contributed to the autosomal recessive manner of the disease. Careful consideration of this event by clinical confirmation of parental carrier status may help to accurately determine the risk of occurrence of this disease in future offspring. Additionally, WES is recommended as a powerful tool to assist in identifying potentially causative variants for heterogeneous diseases such as MDC1A.

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Introduction

Merosin deficient congenital muscular dystrophy type 1A (MDC1A) or laminin-α2 related muscular dystrophy (OMIM entry, #607855) is one of the most common forms of congenital muscular dystrophy (CMD), and accounts for 30% of all cases of CMD in European countries (1). CMD may appear during early onset or later in a patient's life, and is characterized by proximal weakness, contractures, delayed motor development, white matter abnormalities and spiral scoliosis (2,3). CMD is a hereditary disorder caused by recessive mutations in the *LAMA2* gene, which is located on chromosome 6q22-2, spans over 260 kb and is comprised of 65 exons. *LAMA2* encodes the laminin-α2 chain which attaches with laminin-β1 and laminin-γ1 chain to form the heterotrimeric protein laminin 211. Laminin 211 is a primary component of the skeletal muscle basement membrane and the extracellular matrix (1,4,5). The protein interacts with other matrix macromolecules and contributes to cell differentiation, cell movement and tissue phenotypes (4). The majority of reported genetic variations of MDC1A are homozygous or compound heterozygous variants (6,7). *De novo* variations in contrast are not common events and only a few have been reported in patients with MDC1A (8-10).

It is estimated that the prevalence of MDC1A is 1-9/1,000,000 individuals, and accounts for 1-6% of all CMD cases (1,2). MDC1A is more common in Caucasians and rarer in the Asian population (2). However, the prevalence of the disease in the Asian population may be higher than expected due to a lack of widespread availability of appropriate diagnostic testing (11). In the present study, the case of a Vietnamese male child exhibiting clinical signs of muscular dystrophy is reported. The child was previously undiagnosed due to his rare clinical presentation and a lack of appropriate testing. Whole exome sequencing (WES) was performed on the patient and his parents. WES showed that the proband harbored two variants in the *LAMA2* gene. Genotype-phenotype analysis suggested that the patient had classical early onset MDC1A. Additionally, the *de novo* variant was suggested to serve a contributive role in the development of the disease. To the best of our knowledge, the present study is the first to report a case of MDC1A in a Vietnamese patient.

Patients and methods

Sample collection. Prior to collection of samples, written informed consent form was obtained from the parents for research collection and use of biological samples (blood and muscle) and clinical information including the results of the magnetic resonance imaging (MRI) scan, muscle biopsies and genetic testing. The present study was approved by the Institutional Ethical Review Board of Vinmec International General Hospital (Hanoi, Vietnam). From both the patient and his parents, ~2 ml of peripheral blood was collected. Blood samples were stored in EDTA containing tubes at -80˚C. The deltoid was chosen as the muscle to obtain a biopsy from, and a standard procedure was followed (12).

Muscle pathological analysis. To examine the morphology of the muscle, muscle tissue sections were obtained, and were fixed and stained using hematoxylin and eosin staining, as described previously (13). Needle electromyography (EMG) was performed at the quadriceps and gastrocnemius muscle using a Nicolet EDX system (Natus Medical, Inc.).

WES. Genomic DNA was extracted from peripheral blood lymphocytes using a DNA Mini Blood Isolation kit according to the manufacturer's protocol (Qiagen GmbH). A total of 50 ng genomic DNA was used for library construction using a Nextera Rapid Capture kit (Illumina, Inc.) according to the manufacturer's protocol. Paired-end sequencing with a read length of 75x2 bp was performed using a HiSeq 4000 (Illumina, Inc.).

Variant identification and analysis. Burrows Wheeler Aligner (14) was used to map short reads to the human reference genome (GRCh37). Platypus program version 0.8.1 (15) and Genome Analysis Toolkit version 3.6 (16,17) were both used for variant calling. Variants with a minor allele frequency >1%, as reported in the 1000 Genomes Project database (18), were removed. SnpEff program version 4.3g (19) and the Human Genome Variant Database (20) were used for variant annotation. PolyPhen-2 (21) and PROVEAN version 1.13 (22) were both used to predict the impact of missense variant. PolyPhen-2 presents the damaging score as a value between 0 and 1, where a score closer to 1 indicates a high probability of the substitution being damaging. For PROVEAN, if the score is ≤-2.5 (which is used as a threshold), this indicates the variant is damaging. Protein structure analysis was performed using HOPE (23).

Sanger validation. The candidate variants from WES were confirmed using Sanger sequencing on an ABI 3500 DX system with BigDye Terminator version 3.1 (Thermo Fisher Scientific, Inc.). The primer sequences used for validation were: NM_000426.3:c.1964T>C forward, 5'-TGAGGGTGG AGGATACAAATATAG-3' and reverse, 5'-AGGGCTCCGTT CTTATCTGC-3'; and NG_008678.1:c.3556-13T>A forward, 5'-AAGATTTACGTCCTGCCATGC-3' and reverse, 5'-CCA TGTGGGCAACAATCTCTG-3'.

Results

Case presentation. The proband was a male, born by caesarean section at 36 weeks of gestation to healthy and non-consanguineous Vietnamese parents with no family history of any inherited disease. His weight was 3.4 kg and was considered as of no clinical concern at birth. The boy was reported to show a delay in motor milestone acquisition during his life. When visiting our hospital, the proband was 14 years old and he presented with gross motor developmental delay, severe spiral scoliosis and limb-girdle muscular dystrophy. He showed normal intelligence for his age, without any sign of ophthalmoplegia or elongated

Table I. Clinical features of the patient.

Age of onset	At birth
Age when diagnosed	14 years old
Sex	Male
Ethnic group	Kinh Vietnamese
Height	146 cm
Weight	44.2 kg
Brain MRI	WMH on T2-weighted image
Mental retardation	No
Independent ambulation	No
Facial dysmorphy	No
Motor milestone	Sat supported
Contractures	Yes
Scoliosis	Yes
EMG myopathic changes	Yes
Creatine kinase	126 U/l[a]

[a]Reference for merosin deficient congenital muscular dystrophy type 1A <170 U/l. WMH, abnormal brain white matter hyperintensity on T2W; EMG, electromyography; MRI, magnetic resonance imaging.

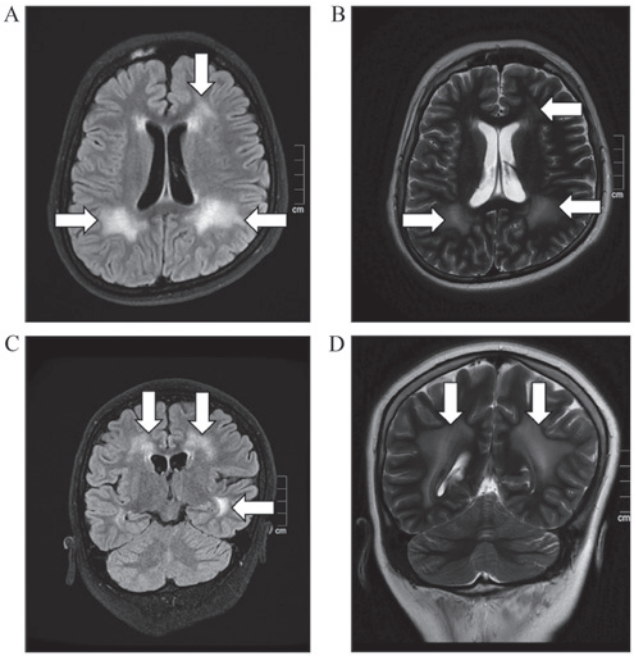

Figure 1. Magnetic resonance imaging results of the patient at the age of 14 years old. (A) Axial FLAIR. (B) Axial T2-weighted image. (C) Coronal FLAIR image. (D) Coronal T2-weighted image. Empty arrows present the brain lesions. FLAIR, fluid attenuated inversion recovery image.

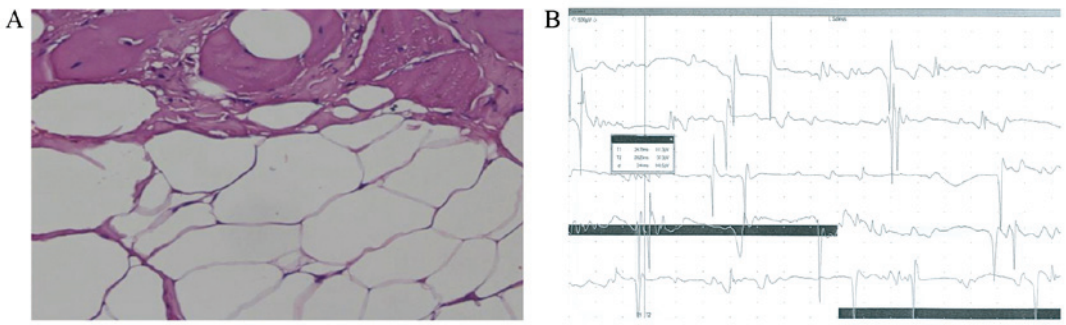

Figure 2. Muscle pathological analysis. (A) Hematoxylin and eosin staining of a muscle biopsy from the deltoid. Magnification, x400. (B) Morphology and recruitment pattern of motor unit action potential.

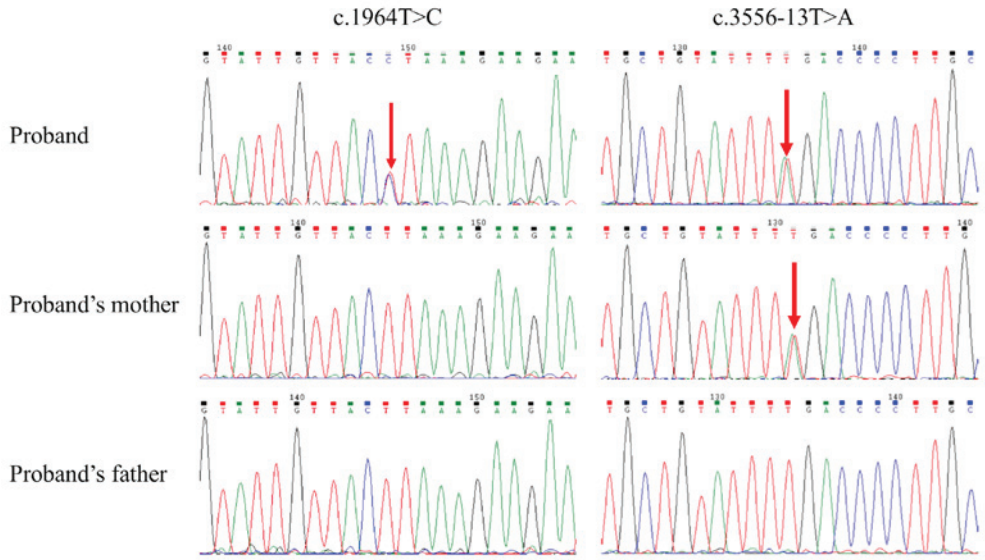

Figure 3. *LAMA2* gene sequence of the proband and the parents. The missense variant, NM_000426.3:c.1964T>C, was only observed in the proband. The intronic variant, NG_008678.1:c.3556-13T>A, was observed in the proband and the mother. Red arrows show the position and heterozygosity of the variation.

face and could speak normally. The creatine kinase (CK) levels were normal (126 U/l; Table I). The boy was not able to sit or ambulate unsupported. A brain MRI scan revealed an increased signal at the frontal and the bilateral occipital lobe. Diffuse brain white matter hypointensity was also observed (Fig. 1). Deltoid muscle biopsy indicated fibrous-adipose replacement (Fig. 2A). In addition, needle EMG showed the motor units exhibited clear characteristics of myopathy (Fig. 2B) (24).

To evaluate gross motor functions, several tools, including the Gross Motor Function Classification System (GMFCS) (25,26), Gross Motor Function Measure 88 scale (GMFM-88) for children with cerebral palsy aged 12-18 years old (27), and the modified Ashworth Scale were used to measure muscle spasticity (28). The proband was scored at level V based on the GMFSC scale, meaning that he had severe limitations and entirely relied on a wheelchair for movement. GMFM-88 evaluation showed that the patient achieved 38 points in domain 'Lying and Rolling', 31 points in domain 'Sitting', one point in domain 'Crawling and Kneeling', and zero points in domains 'Standing', 'Walking', 'Running' and 'Jumping'. Modified Ashworth evaluations indicated that the scores of the upper and lower limbs ranged from 3-4 points, indicating that there was a considerable increase in muscle tone, and rigidity in flexion or extension.

Genetic studies. A total of 110, 86 and 101 million paired-end reads were obtained from the proband, and the father and the mothers genomes, respectively, where 95% of the reads had a Phred-score ≥30 (95% of the reads were called with a correct probability ≥99.9%) (29). Average coverage at the targeted regions were 81X, 66X and 80X for the proband, father and mother, respectively. Two variants were detected in the *LAMA2* gene (Reference sequence: NM_000426.3) of the proband and these variants were not previously described in the Vietnamese genetic variation database (genomes.vn/) (30) indicating their rare frequency in the population. Of these, the missense variant, NM_000426.3:c.1964T>C, p.Leu655Pro, located in exon 14, resulted in a substitution of leucine to proline at the 655th amino acid residue. It appeared as a *de novo* variant as it was not observed in the parents. This variant has been not listed in the Leiden Open Variation Database (databases.lovd.nl/shared/genes/LAMA2) or elsewhere suggesting its novelty. In addition, WES results indicated that the proband and his mother carried a splice site variant (NG_008678.1:c.3556-13T>A) which was present

at the intron-exon boundary of exon 25 of the *LAMA2* gene. Sanger sequencing confirmed the findings from WES where the missense variant (NM_000426.3:c.1964T>C) was only detected in the proband; whereas the splice site variant (NG_008678.1:c.3556-13T>A) was found in the proband and his mother, but not in the father (Fig. 3). The splice site variant has been previously observed in two Chinese patients with MDC1A and is reported to cause mRNA splicing (6).

In silico predictions. PolyPhen-2 predicted that the missense variant (NM_000426.3:c.1964T>C) was damaging with a score of 1. PROVEAN predicted that this variant was 'deleterious' with a score of -6.992. Additionally, protein structure analysis using HOPE suggested that the missense mutant, the size of which was smaller compared with the wildtype, may result in a loss of amino acid interactions. Thus, a substitution of leucine to proline may disturb a functional domain of the laminin protein and abolish the proteins function.

Discussion

In the present study, two variations in the *LAMA2* gene of a male proband who exhibited clinical manifestations of CMD were identified. The missense variant (NM_000426.3:c.1964T>C) is located in the N-terminal domain of laminin-α2. A previous study found that half of the variants detected in a cohort of 43 patients with MDC1A were located in the N-terminal domain (6). Therefore, this may suggest that this domain is either more vulnerable than the other domains to mutations, is more sensitive to mutations or that it exhibits a crucial function on the part of the protein. WES analysis of the proband and his parents alongside database analyses indicated that NM_000426.3:c.1964T>C was *de novo* and a novel variant. A splice site variant (NG_008678.1:c.3556-13T>A) in the proband and his mother was also detected. This variant and its mRNA splicing effect (premature termination of transcription) have been previously described in two Chinese patients with MDC1A (6).

Together, these two variants likely caused a deficit in laminin-α2 function, chain which is a primary component of a trimeric basement membrane glycoprotein (1,4,5). Mutations in the *LAMA2* gene result in defective function of the laminin protein with consequential poor muscle fiber adhesion and degeneration (31). Phenotype-genotype analysis indicated that this proband was associated with classical early onset LAMA2-associated muscular dystrophy. Therefore, these two variants were submitted to the Global Variome Shared LOVD database (databases.lovd.nl/shared/individuals/00208524).

The missense variant was searched against in multiple databases and was not found to have been previously reported. Therefore, the present case report may be the first report underlining genetic variations in a Vietnamese individual with MDC1A. In addition to the rare nature of MDC1A, a lack of common symptoms in the child, such as increased levels of CK and ophthalmoplegia, and the genetic heterogeneity of this disease made diagnosis difficult. CK levels are often elevated in patients with MDC1A during the early years of a patient's life and tend to decrease with age (32). The proband in the present report exhibited normal levels of CK at the age of 14. Several studies have reported normal CK levels in patients with MDC1A (6,11,33). Therefore, age should be taken into account when interpreting the CK level in each patient with CMD (34). In the present study, the patient was suspected to suffer from X-linked adrenoleukodystrophy owing to his abnormal brain MRI scan. Genetic testing of the *ABCD1* gene, defects of which cause X-linked adrenoleukodystrophy were performed. However, there was no evidence of mutations found and the patient remained undiagnosed prior to WES testing (data not shown).

MDC1A exhibits a wide spectrum of genetic and clinical heterogeneity. Immunohistochemistry (IHC) is a first-tier test used to determine CMD-associated diseases. Commercially available antibodies, such as clone 5H2, clone Mer3/22B2 and clone 4H8-2 are available for performing laminin protein assays (7). However, as MDC1A is extremely rare in Asian populations, including the Vietnamese, IHC using these specific antibodies for laminin-α2 is often not viable. Therefore, in addition to clinical features (phenotypes, brain MRI, EMG and muscle morphology), molecular genetic testing, such as WES, is highly recommended (7,35). Additionally, WES should be performed for both the patient and their parents, as it assists in improving our understanding of the diagnostic yield for genetically heterogeneous disorders (36), and may result in the identification of novel variants which contribute to this disease (37). With the increase in the use and availability of next generation sequencing techniques, novel variants/mutations associated with MDC1A have been uncovered (7). The newly discovered variant in the present study adds to the genetic spectrum of the disease, and also suggests the contribution of a *de novo* event to the autosomal recessive inheritance of MDC1A. Therefore, *de novo* events should be taken into consideration to accurately determine the occurrence risk of this disease in future offspring.

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Availability of data and materials

Data containing information on the variants were deposited on the Global Variome Shared LOVD database (databases.lovd.nl/shared/individuals/00208524). The datasets used or analyzed in the present study are available from the corresponding author on reasonable request.

Authors' contributions

KTT prepared the experiments and wrote the manuscript. VSL performed the bioinformatics analysis. LTN and CDV performed the clinical diagnoses. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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