Exome Sequencing Reveals Diagnosis of LAMA2-Muscular Dystrophy and Possibility of Coexisting Bethlem Myopathy in a Neonate

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Abstract

We reported a neonate presenting with muscle weakness, hypotonia, and joint contractures since birth. Investigations revealed significantly elevated creatinine-phosphokinase, abnormal electromyography suggestive of muscle disease and normal magnetic resonance imaging (MRI) of the brain. Exome sequencing revealed homozygous pathogenic mutations in *LAMA2* (NM_000426.3: c.7881T > G, p.(His2627Gln)) and a heterozygous likely-pathogenic mutation in *COL6A2* (NM_001849.3: c.1970–2A > G). Parental segregation by Sanger sequencing confirmed a heterozygous carrier state for the *LAMA2* variant in both parents, thus confirming the diagnosis of autosomal recessive LAMA2-muscular dystrophy (LAMA2-MD) in the proband. The *COL6A2* variant segregated with the as-yet asymptomatic mother. Musculoskeletal MRI of the proband at 12 months of age revealed peripheral involvement of the vastii, rectus femoris, gastrocnemius and the soleus, with relative central sparing, without areas of fatty infiltration; not serving to distinguish clearly between LAMA-MD and COL6A2- related disease. Reverse phenotyping of a 27-year-old mother revealed a normal musculoskeletal MRI and clinically absent red flags. Potential explanations for the heterozygous likely-pathogenic *COL6A2* variant in the proband and the mother include (a) a coexisting diagnosis of autosomal dominant COL6A2-related myopathy, likely Bethlem myopathy, which has a variable clinical phenotype and age of onset; (b) a carrier state for autosomal recessive Ullrich congenital muscular dystrophy; or (c) a heterozygous *COL6A2* variant contributing as a synergistic factor along with homozygous *LAMA2* mutation. The couple was offered genetic counseling regarding the proband and the future pregnancies.

Keywords

- next-generation sequencing
- arthrogryposis
- muscle disorders
- genetic counseling
- dual diagnosis

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Introduction

Next-generation sequencing (NGS) is enabling new insights into the under-recognized prevalence of dual/multiple genetic diagnoses,[1,2] and outlining cases of complex oligogenic/multigenic inheritances due to synergism of pathogenic variants in >1 gene, in the same individual.[3] We present a newborn with a diagnosis of LAMA2-muscular dystrophy (LAMA2-MD) and possibly coexisting COL6A2-related Bethlem myopathy (BM).

Case Presentation

We presented a female neonate, a first-born to nonconsanguineous parents of Asian descent. Reduced fetal movements were documented in the antenatal period. The patient was delivered vaginally at term, with normal Apgar scores. The birth weight, length, and head circumference were 2,900 g, 48 cm, and 33 cm, respectively. The child had stable vital signs and normal respiratory effort. She was noted to have extreme floppiness and paucity of spontaneous movements in all limbs. There was no feeding difficulty, fatigability, or diurnal variation in the weakness. Examination revealed hypotonia, hyporeflexia, and generalized weakness, sparing the facial and ocular muscles. Her cranial nerve examination was normal. She had midface hypoplasia, microretrognathia, mild macroglossia, and congenital contractures at multiple joints *including* bilateral shoulder, wrist, metacarpophalangeal, hip, knee, and ankle joints (►**Fig. 1**). There were no fasciculations, neurocutaneous markers, or skin laxity.

The creatinine phosphokinase on day 12 of life was significantly elevated (2819 IU/L, upper-limit 180 IU/L). Needle electromyography in the right-deltoid and bilateral vastii revealed absence of any spontaneous activity and small polyphasic motor unit potentials, suggesting a generalized muscle disease. Nerve conduction study, MRI brain, two-dimensional echocardiography, ophthalmic and hearing evaluation, performed in the neonatal period, were normal. She had difficulty gaining weight despite adequate feeding (2800 g on day 24). Pedigree details and parental examinations were unremarkable. Our clinical suspicion included the congenital muscular dystrophy group of disorders.

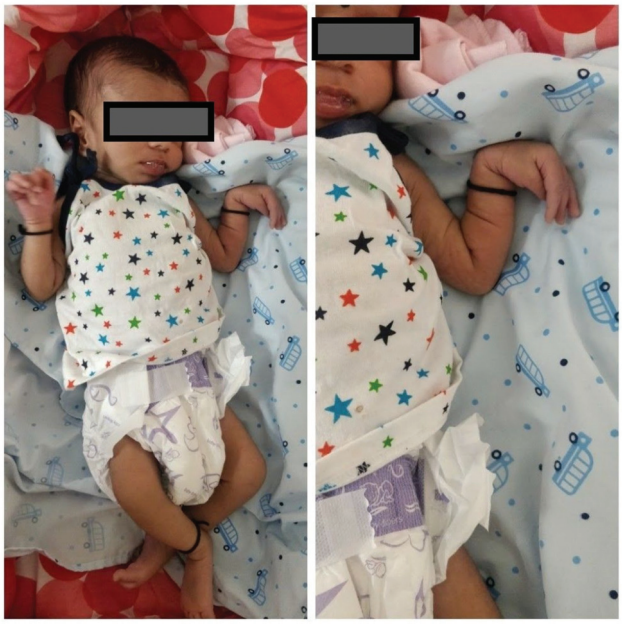

Fig. 1 Clinical picture of the neonate. The contracture at the wrist and the knee joints is evident in the picture. Also note microretrognathia and mild macroglossia.

NGS-based exome sequencing (ES) was ordered on day 43 of life. The sequencing platform used was Illumina Nextseq 550. It revealed pathogenic homozygous mutations in *LAMA2* (c.7881T > G, p.His2627Gln) and a heterozygous, likely-pathogenic mutation in *COL6A2* (c.1970–2A > G) (►**Fig. 2**). The depth of the sequencing was 58x (*LAMA2*) and 78x (*COL6A2*), respectively. The variant classification was as per American College of Medical Genetics and Genomics) guidelines and Varsome database.[4,5] Combined Annotation Dependent Depletion scores of the two variants were 3.606 (*LAMA2*) and 32 (*COL6A2*), respectively.[6] The minor allele frequency was reported in the gnomAD to be 0.00003189 for the COL6A2 variant, but was not reported for the *LAMA2* variant. The *LAMA2* variant had been reported in a homozygous state with complete absence of merosin.[7] Its predicted effect was to disrupt the evolutionarily conserved histidine amino-acid, present in G-domain of laminin, which plays an important role in linking the extracellular matrix and the sarcolemmal cytoskeleton. The *COL6A2* variant has not been reported in the literature; however, the variant was detected in the splice-site and was predicted to disrupt the highly conserved acceptor splice-site (AG nucleotide) of intron-25. In-silico analysis by MutationTaster predicted the mutation to be damaging and splice-site predictor tool dbscSNV for splicing consensus regions showed a higher score (ADA 0.99 and RF 0.87) indicating the detected splice-site variant was predicted to be highly damaging.[8]

Parental segregation by Sanger sequencing was performed when the baby was 11 months old. It confirmed the heterozygous carrier state for the *LAMA2* mutation in both parents (►**Fig. 2**), which supported a diagnosis of autosomal recessive (AR) LAMA2-MD (OMIM #607855) in the proband. The *COL6A2* variant segregated with the as-yet asymptomatic mother (►**Fig. 2**); the interpretations being not as straightforward to deduce. It necessitated a relook into the phenotypes of the mother and child.

At 12-month clinical follow-up, proband was noted to have delayed motor milestones. She had not yet achieved independent head support, rolling over, sitting supported, or reaching for toys. Social and language milestones were age-appropriate. The mother denied feeding difficulties. Poor growth was noted: weight 5.3 kg (World Health Organization [WHO] growth chart <-3 Z-score), length 70 cm (WHO growth chart -1 Z-score), and head circumference 44 cm (WHO growth chart between 0 and -1 Z-score). The child had a paucity of spontaneous movements; however, antigravity movements were possible in all four limbs. Hypotonia and contractures remained unchanged.

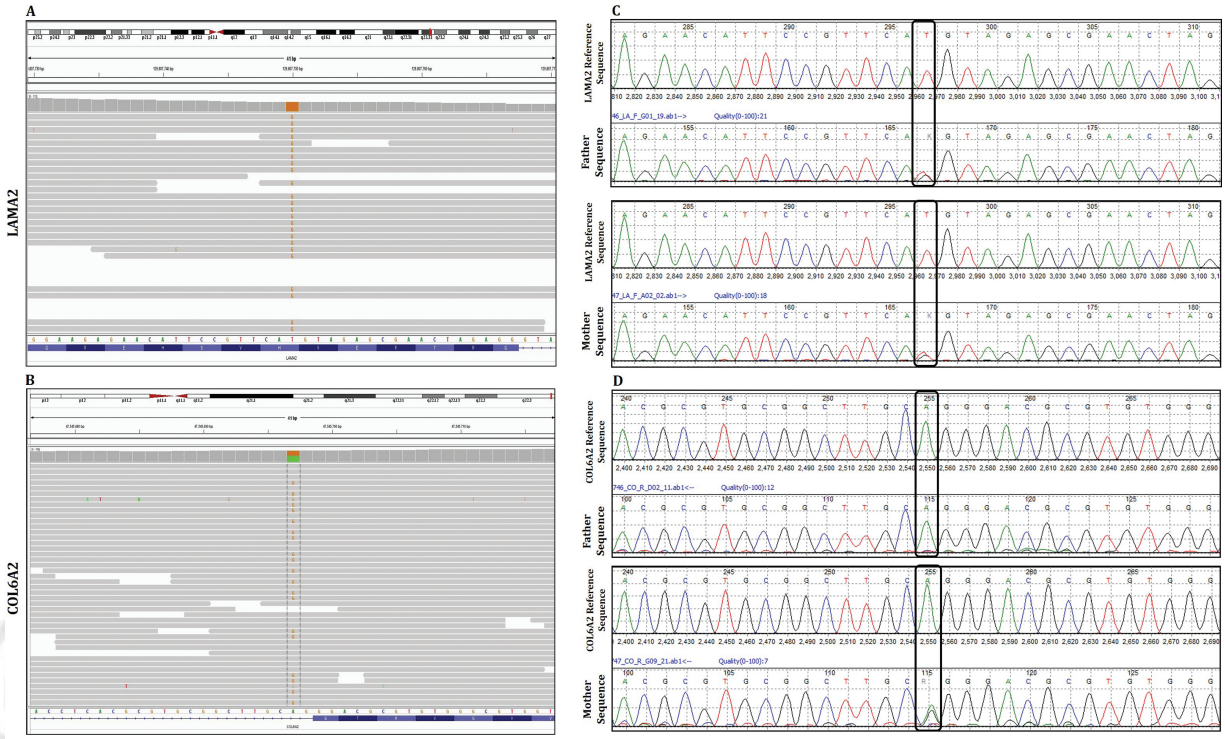

Fig. 2 Integrative genomics viewer (IGV) and Sanger electropherogram images of *LAMA2* and *COL6A2* gene variants identified in the family: The IGV image depicts variants identified in the child. (**A** and **B**) and Sanger image is showing the parental target variant status (**C** and **D**). (**A**) A homozygous missense variant c.7881T > G (chr6: 129807750T > G) in *LAMA2* and (**B**) a heterozygous splice site variant c.1970–2A > G (chr21: 47545697A > G) in *COL6A2* gene is shown in the above image for child against human genome reference sequence GRCh37/hg19. (**C**) A heterozygous variant c.7881T > G (GRCh37/hg19, chr6: 129807750T > G) is pictured in *LAMA2* gene in both paternal and maternal samples. (**D**) Variant c.1970–2A > G (GRCh37/hg19, chr21: 47545697A > G) in *COL6A2* gene is shown in a heterozygous state in mother's sample and is not detected in father's sample. The target variant is marked with the black line box for both genes and in both samples. Mutation surveyor software version 5.1 (Softgenetics, State College, Pennsylvania United States) is used for identifying the variants. LAMA2, laminin subunit α 2, COL6A2, collagen type VI alpha 2 chain.

To delineate the proband's phenotype further, limited musculoskeletal MRI was conducted at 12 months. It revealed peripheral involvement of the vastii, rectus femoris, gastrocnemius, and the soleus, with relative central sparing, without areas of fat replacement (►**Fig. 3**). These changes were nonspecific, and were not helpful in distinguishing between LAMA2-MD and COL6A2-related disease (suspected, BM). An elaborate musculoskeletal MRI and repeat MRI brain were deferred due to the potential risks associated with elective anesthesia. Reverse phenotyping of the 27-year-old mother revealed normal musculoskeletal MRI and clinically absent red flags (►**Supplementary Fig. S1**).

The potential implications of the heterozygous likely-pathogenic *COL6A2* variant in the proband and her mother were to cause: (a) a coexisting autosomal dominant (AD) BM (OMIM #158810), which is known to be clinically diverse, with variable ages of onset; in the mother as well as the child; (b) an asymptomatic carrier state for AR Ullrich congenital muscular dystrophy (UCMD), an allelic disorder of BM; or (c) a probable synergistic heterozygosity of the *COL6A2* variant along with *LAMA2* variant.

The family was offered genetic counseling regarding the spectrum and prognosis of the two conditions. Appropriate occupational therapy was instituted. She received respiratory vaccines in addition to routine vaccines. Clinical surveillance in the mother was emphasized. Risk of recurrence for LAMA2-MD for future pregnancies was outlined as 25%. A 50% chance of the future sibling inheriting the *COL6A2* variant was discussed; however, prediction of its impact on the phenotype could not be precisely determined.

Discussion

CMDs are a genetically heterogenous group of around 30 disorders resulting in a spectrum of overlapping clinical features involving progressive muscle weakness, floppiness, and joint contractures.[9] These disorders can have different ages of onset, patterns of muscle involvement, severity, and inheritance patterns.[9]

Among the CMDs, LAMA2-MD (merosin-deficient) is reported to be the commonest type, accounting for nearly one-third of all cases.[10] Mutations in *COL6A2* are known to be associated with AD BM as well as AR and (rarer) AD forms of UCMD.[11,12] BM is a usually a milder form of the disease with a wide spectrum of ages of onset and severity.[11,12] The clinical spectrum of neonatal onset LAMA2-MD and COL6A2-related BM is summarized in ►**Table 1**.[11–15]

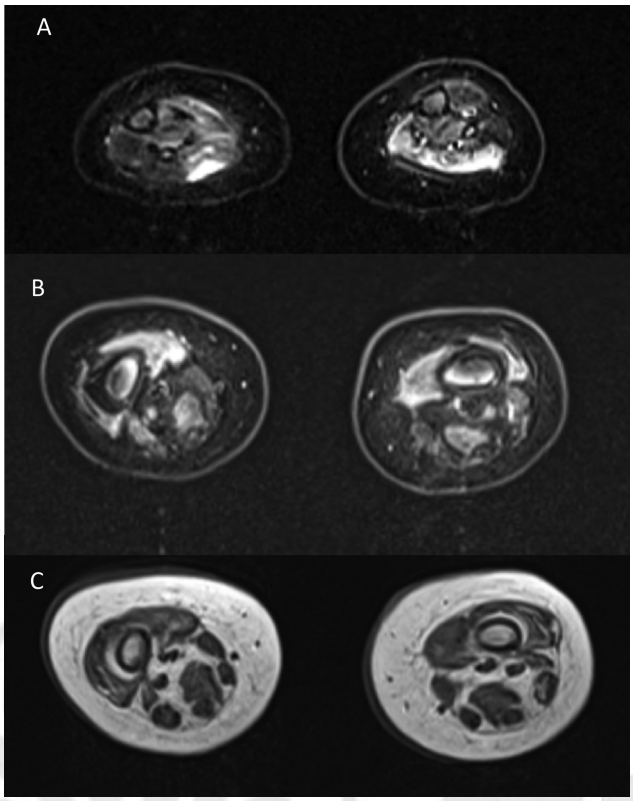

Fig. 3 Musculoskeletal magnetic resonance imaging of the lower limb muscles in the proband. (**A**) Short inversion time inversion recovery (STIR) axial images through the calf show peripheral involvement of the gastrocnemius and soleus muscles bilaterally (slightly asymmetric). (**B**) STIR and (**C**) T1 axial images through the thigh show signal abnormality predominantly in the periphery of the vastii and quadriceps muscles, with relative sparing of the central portions. The remaining thigh muscles are relatively uninvolved.

Similarly, a *COL6A2* variant, c.1970–1G > C, has been reported as one of the compound heterozygous variants in a severe AR UCMD case; while the other publications have reported similar heterozygous variants in this intronic region to cause milder symptoms of BM with late onset.[16,17] The nature of the *COL6A2* variant prompted us to consider one probable explanation as a codiagnosis of BM in the child, inherited as an AD trait from the mother. The alternative explanation included an asymptomatic *COL6A2* carrier state in the mother-child duo, for the AR form of UCMD. While the overlap with LAMA-MD may make phenotype appreciation of BM difficult in the proband, a close clinical follow-up of the mother may help us resolve this clinical uncertainty with time.

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We postulate a third possibility in which the heterozygous *COL6A2* variant is acting synergistically with the *LAMA2* variant, resulting in a digenic inheritance. In a series of 207 individuals with suspected inherited myopathy from the Indian subcontinent, 13 cases (6.28%) had pathogenic variants in >1 gene, suggesting a high incidence of synergistic heterozygosity in genetic myopathies.[3] While in some of

Table 1 Salient clinical features of neonatal-onset LAMA2-muscular dystrophy and COL6A2-related Bethlem myopathy

	LAMA2-muscular dystrophy (OMIM #607855)	COL6A2-related Bethlem myopathy (OMIM #158810)
Gene	*LAMA2*	*COL6A2*
Inheritance	Autosomal recessive	Autosomal dominant
Spectrum of onset	• Severe, neonatal onset, congenital muscular dystrophy (also abbreviated as, MDC1A, our case) • Milder, childhood to late adulthood onset disease	• Prenatal • Neonatal • Early childhood • Adulthood/late adulthood (4^{th} to 6^{th} decade)
Clinical features		
Musculoskeletal	Features of neonatal onset disease • May be associated with prenatal onset reduced fetal movements* • Severe neonatal onset hypotonia* • Neonatal onset muscle weakness* • Joint contractures—initially shoulder, elbow, hip, knee joints* • Joint contractures—progressively involving temporomandibular joint, cervical spine and distal joints • Delayed motor milestones* • Most children are nonambulatory	Clinical variability known • Prenatal onset—decreased fetal movements* • Neonatal onset—paucity of movements*, joint contractures*, hypotonia* or torticollis • Early childhood: delayed motor milestones, contractures that may be transient and self-resolving. Evolving disabling contractures of the fingers, wrists, elbows, and ankles • Adulthood: proximal muscle weakness and atrophy, progressive ankle and long finger flexor contractures. Progressive scoliosis

Table 1 (*Continued*)

	LAMA2-muscular dystrophy (OMIM #607855)	COL6A2-related Bethlem myopathy (OMIM #158810)
	• Most achieve independent sitting, few (15%) achieve independent ambulation • Weakness of neck flexor muscles, axial weakness • Progressive scoliosis/kyphoscoliosis • Evolving facial weakness, temporomandibular joint weakness • Macroglossia* • Ophthalmoparesis (usually by 2 years)	and stiffness of the back (above features absent in the mother)
Intellect	• Usually normal • Rarely (<7%) cognitive impairment	Normal
Other features	• Failure to thrive* • Swallowing difficulties, frequent aspirations, gastroesophageal reflux, recurrent respiratory tract infections, evolving restrictive lung disease, respiratory failure • Seizures (30%) • Cardiac involvement—rare	• Evolving respiratory and diaphragmatic muscle weakness, necessitating nocturnal ventilation (usually by adulthood) • Cardiac involvement—rare
Common differential diagnosis	• Other congenital muscular dystrophies • Congenital myasthenic syndromes • Congenital and metabolic myopathies • Spinal muscular atrophy	
Investigations		
Creatinine phosphokinase	Usually more than four times the normal upper limit. Reduces later as the disease progresses	Normal or only mildly elevated
MRI	• Brain—diffuse abnormal white matter signals, neuronal migration defects, structural brain malformation (absent in our case. Follow up MRI brain at 6–9 months could not be performed in our case) • Musculoskeletal—involvement of anterior thigh muscles common* Usually associated with fatty infiltration Sparing of the gracilis, sartorius, vastus medialis, and rectus femoris muscles (features may overlap COL6A2-related myopathies)	• Brain—usually normal • Musculoskeletal—Thigh: frequent and significant involvement of the vastii Peripheral rim of involvement with central sparing relatively* Classic abnormal signal in the central portion of rectus femoris (called as "central cloud phenomenon"). Calf muscles: peripheral rim of abnormal signal in soleus and gastrocnemius*
Electromyogram	Myopathic pattern	Myopathic pattern

Abbreviations: MCD1A, merosin-deficient congenital muscular dystrophy type-1A, MRI, magnetic resonance imaging.
Features that were present in the current case are marked with an asterisk (*).[11–15]

these cases, both the genes were expressed phenotypically (*viz* dual diagnosis); in some instances the 'synergism' of > 1 genetic mutations resulted in a net-amplified expression of one trait preferentially over the other (i.e facilitation).[3] Immunohistochemistry studies assessing the laminin α2 and collagen VI expression of the dermal fibroblasts through skin biopsy could not be obtained in this case, but could potentially further clarify the impact of the COL6A2 variant in the future.[12,13] Despite overlapping features, a future musculoskeletal MRI in the child and her mother may help to establish the phenotype; fatty infiltration of the muscles is relatively more common in LAMA2-MD, while a "central-cloud phenomenon" in rectus femoris is classic for BM (►**Table 1**).[14,15]

NGS-based tests, including targeted panels and ES, have evolved over the last 15 years, and have enabled more precise molecular diagnoses in neuromuscular disorders.[18] In a recent study describing the application of ES in 190 individuals with neurological disorders, the diagnostic rate (DR) of the cohort was 56%; while the subgroup of muscle disorders ($n = 18$) had a higher DR (60%).[18] Lately, adding to the diagnostic prowess of NGS is its ability to diagnose dual and supernumerary genetic diagnoses, as highlighted by the series on Indian myopathies above.[3] In a large retrospective analysis of 7,374 adults undergoing NGS tests, a definite diagnosis was established in 2,076 (DR: 28.1%), with 101 individuals (4.9% of those with a diagnosis, 1.4% of all patients) harboring multiple genetic diagnoses.[2] While 97 among those had dual genetic diagnoses, three patients had three genetic disorders each, and one had four distinct molecular diagnoses.[2] Similar results were reported in the study of 2,000 exomes by Yang et al (DR: 25%); 23 patients

(4.6% of those with a diagnosis, 1.4% of all patients) received dual genetic diagnoses.[3] With increasing application of NGS in clinical practice, we expect more cases with similar results to emerge, thereby guiding future research in understanding complex disease inheritances, interactions, and pathophysiology.

The implications of recognizing underlying multiple genetic diagnoses are manifold.[2,19] A complete genetic diagnosis enables comprehensive prognostication and surveillance of the patient as well as the as-yet asymptomatic at-risk relatives.[19] In this case, the genetic results alerted us to follow the mother clinically, and explain the importance of doing so even after her 6th decade. Comprehensive genetic diagnoses also enable robust prenatal counseling for future pregnancies,[19]although in our case the significance of the *COL6A2* variant remains unclear (at least at this point in time). In the current case, NGS established the diagnosis of LAMA2-MD, and a codiagnosis of BM remains suspected. The case, however, does highlight the emerging potential of NGS in uncovering complex inheritances of clinically overlapping disorders.

Conclusion

ES has the potential to establish dual genetic diagnoses, which can have important clinical implications for the patient as well as the family.

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None.

Conflict of Interest
None declared.

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