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Case report

Clinical and neuroimaging findings in two brothers with limb girdle muscular dystrophy due to *LAMA2* mutations

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Abstract

Recessive mutations in *LAMA2* commonly cause congenital muscular dystrophy (MDC1A) and, rarely, limb girdle muscular dystrophy (LGMD). We report 2 brothers who presented in adulthood with LGMD due to novel mutations in *LAMA2* identified by whole exome sequencing (WES). Muscle biopsy more than 30 years ago demonstrated dystrophic changes but was not available for immunoanalysis. Muscle MRI demonstrated involvement of peripheral muscle with internal sparing classically seen in collagen-VI related disorders. Extensive genetic testing, including *COL6A1/2/3*, was performed prior to WES. Subsequent skin biopsy immunoanalysis demonstrated laminin α2 partial absence. The phenotype of the patients was notable for novel central nervous system findings, namely bilateral signal changes in the globi pallidi, and presence of dilated cardiomyopathy (DCM). They also illustrate the similarity in muscle MRI in collagen VI and laminin α2-related disorders, both of which are due to mutations in genes encoding extracellular matrix proteins.

Keywords: Limb girdle muscular dystrophy; Laminin α2; Collagen VI; Muscle MRI

1. Introduction

Recessive mutations in *LAMA2*, encoding the extracellular matrix protein laminin α2, a subunit of laminin 2 (merosin, laminin 211), the most abundant laminin isoform in the basement membrane of differentiated skeletal muscle fibres, typically cause a severe congenital muscular dystrophy with complete absence of laminin α2 on immunoanalysis (MDC1A) [1]. Less commonly *LAMA2* mutations may present with a milder phenotype sometimes with limb girdle muscular dystrophy (LGMD) [2,3], although this is speculated to be an underdiagnosed cause of LGMD [4]. This milder presentation is associated with reduced rather than absent laminin α2 expression levels on muscle biopsy [5].

Laminin α2 is expressed in cardiac and skeletal muscles as well as both the central and peripheral nervous systems and skin [6]. In addition to skeletal muscle pathology, associated features in *LAMA2* related disease include cerebral white matter changes and, less commonly, structural brain abnormalities [7], which may be associated with central nervous system symptomatology, such as intellectual disability or seizures, in a minority of patients [8]. Patients are also reported with peripheral neuropathy [9,10]. Subclinical cardiac involvement has been observed in a small proportion of MDC1A patients [5,8,11], and laminin α2 together with the dystrophin glycoprotein complex has been shown to co-localise to the cardiac muscle sarcolemma and transverse tubules [12]. In *LAMA2* related LGMD, dilated cardiomyopathy (DCM) has been reported in two patients [13,14], although it is unclear if this is a direct consequence of *LAMA2* mutations or a coincidental finding.

Muscle MRI imaging in *LAMA2* related disease has not been widely reported. Signal abnormalities in the adductor magnus and biceps femoris muscles were observed in one patient with *LAMA2* related LGMD [9], and it has also been noted that a pattern of concentric atrophy of muscles similar to that observed in collagen VI-related disorders may be observed in mild cases of laminin α2 deficiency [15].

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LGMDs are highly genetically heterogeneous and overlap clinically with several other muscle diseases including Bethlem myopathy, Emery–Dreifuss muscular dystrophy or myofibrillar myopathies [16]. Standard diagnostic work up includes muscle biopsy with immunoanalysis to assess changes in relevant proteins to direct genetic testing [17]. A minority of LGMD patients do not obtain a diagnosis following these investigations [18,19] although next generation sequencing techniques, such as whole exome sequencing (WES), are likely to reduce this proportion.

Here we present two brothers with LGMD first assessed by our centre 5 years ago. The absence of muscle biopsy immunoanalysis as a result of the biopsy being performed more than 30 years ago hampered obtaining a diagnosis until they underwent WES, which identified novel compound heterozygous mutations in *LAMA2*. Muscle MRI was useful in confirming this diagnosis and illustrates the overlap in MRI findings in disorders with shared pathogenic mechanisms, in this case between disorders associated with extracellular matrix protein abnormalities.

2. Case report

Two brothers born to unrelated parents and with no family history of neuromuscular disorders were assessed in our centre at ages 58 and 56 years respectively. Patient 1, the eldest, had delayed motor milestones and a history of mild difficulties climbing stairs and rising from a chair since childhood. He had Achilles tendon lengthening surgery at 12 years of age. Patient 2 reported being slower than his peers from 7 years of age. On examination, both brothers had a waddling gait, were unable to stand on heels or tip toes, and both had rigid spine and ankle contractures. In addition Patient 1 showed pes cavus bilaterally, and Patient 2 showed mild scapular winging, mild finger flexion contractures, and thigh muscle wasting. Manual muscle testing in both patients demonstrated mild weakness in shoulder girdle and upper arm muscles (Medical Research Council (MRC) grade 4− to 4+), and pronounced weakness in hip extension and abduction (MRC grade 2–3). Both brothers had normal cognitive function, having had careers in management level positions. Patient 1 developed dilated cardiomyopathy, unrelated to ischaemic heart disease, in adulthood. On recent cardiac review, at the age of 63 years he had atrial fibrillation and left bundle branch block; transthoracic echocardiogram demonstrated ejection fraction of 20%. Respiratory function was normal in both patients. Neither had a history of seizures or cognitive deficits.

Serum creatine kinase was approximately 400 u/L in both patients. Neurophysiology in Patient 2 showed evidence of a mixed sensorimotor neuropathy and a chronic myopathic disorder. Patient 2 had a muscle biopsy at age 33 years which was reported to show dystrophic changes. The biopsy is no longer available for immunoanalysis and both patients declined to have a repeat muscle biopsy.

Muscle MRI was performed in both brothers following our assessment (Fig. 1). This demonstrated that the periphery of muscles was most affected with relative sparing centrally, and sparing of the gracillis and the sartorius muscles in the thigh.

Genetic investigations including *DMD* MLPA and sequencing *of LMNA/C*, *CAPN3*, *ANO5*, *FKRP*, *COL6A1*, *COL6A2* and *COL6A3* genes were negative.

WES was then performed in both brothers and compound heterozygous mutations in *LAMA2* (NM_000426.3) were identified: a frameshift variant in exon 32 (c.4533delT, p.(Gly1512fs*)); and a missense variant at a highly conserved position in exon 4 (c.611C>T, p.(Ser204Phe)), which is predicted to be damaging by SIFT, Mutation Taster and FATHMM. Both variants are absent in the Leiden Muscular Dystrophy Mutation Database (http://www.dmd.nl/), and also absent in the Exac database of 62,000 control exomes (http://exac.broadinstitute.org/). Variants were confirmed by Sanger sequencing and both were absent in their unaffected sister. Of note, no variants meeting filtering criteria (MAF <0.01 and Variant Effect Predictor score of moderate to severely damaging) were present in known DCM genes (*MYH7*, *MYBPC3*, *TTNT2*, *TNNI3*, *TPM1*, *ACTC1*, *CSRP3*, *PLN*, *SNC5A*, *TAZ*, *TTN*).

In the absence of muscle biopsy immunoanalysis we obtained skin biopsies from both patients and assessed expression of several laminin chains. This demonstrated a pronounced partial deficiency of laminin α2 (using 2 antibodies) at the epidermal/dermal junction and in sensory nerves (Fig. 2). A deficiency of laminin β1 of unknown significance was also noted (not shown). Expression of laminin α5, β2 and γ1 was similar to controls.

Brain MRI was performed in Patient 1 (Fig. 3). This demonstrated diffuse white matter changes in the cerebral hemispheres on T2 and FLAIR, and increased diffusion on Diffusion Weighted Imaging. There was also bilateral symmetrical increased signal in the globi pallidi. No other abnormalities were noted.

3. Discussion

The genetic heterogeneity of LGMDs can result in a lengthy time to diagnosis for patients, and difficulties in obtaining standard diagnostic tests may contribute to this delay. In this family application of WES enabled targeted diagnostic procedures, namely brain MRI and immunohistochemistry of skin biopsies, to confirm pathogenicity of novel variants in a known disease gene, overcoming the limitation of absent muscle biopsy immunoanalysis and expediting a genetic diagnosis. Interpretation of variants identified by WES in combination with deep phenotyping data, including in this instance muscle MRI, is a powerful approach and may be used to direct further specific investigations to prove or disprove a number of differential diagnoses identified.

The muscle MRI findings were very similar to the muscle MRI pattern associated with Bethlem myopathy caused by mutations in collagen-VI genes [20]. These findings have been considered to be almost pathognomonic for these disorders [15] and yet as our patients' imaging demonstrates that there is a differential diagnosis associated with this pattern, which has been previously noted [15]. The sparing of gracillis and sartorius however is atypical for collagen-VI related myopathy. The similarity in MRI findings between collagen VI-related