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LGMD2D syndrome: the importance of clinical and molecular genetics in patient and family management

Khalid M. Al-Harbi [1] and Atiyeh M. Abdallah [2]

1 Pediatric Department, College of Medicine, Taibah University, Maternity & Children Hospital, Al-Madinah, Saudi Arabia
2 West Midlands Regional Genetics Laboratory, Birmingham Women's NHS Foundation Trust, Birmingham, United Kingdom

Correspondence to: Dr. Atiyeh M. Abdallah
West Midlands Regional Genetics Laboratory
Birmingham Women's NHS Foundation Trust
Mindelsohn Way, Edgbaston, Birmingham, B15 2TG, United Kingdom.
TEL: +447740588741; E-MAIL: atiyeh.abdallah@bwnft.nhs.uk

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Abstract

We report the case of a seven-year-old female from a consanguineous Saudi family with autosomal recessive limb girdle muscular dystrophy type 2D (LGMD2D) most likely caused by a rare SGCA mutation. Histopathological and molecular investigations resulted in the discovery of a homozygous mutation (c.226 C>T (p.L76 F)) in exon 3 of SGCA in the patient. The parents and one sibling were heterozygous carriers, but the mutation was not otherwise detected in 80 ethnic controls from the same geographic area. *In silico* analysis revealed that the mutation resulted in a functional leucine to phenylalanine alteration that was deleterious to the protein structure. This is only the second reported case of the p.L76F mutation in LGMD, and highlights that molecular genetics analysis is essential to deliver the most appropriate management to the patient and offer the family genetic counseling.

CASE REPORT

INTRODUCTION

Limb-girdle muscular dystrophy (LGMD) describes a group of genetic disorders characterized by progressive degeneration of the proximal limb muscles. Clinical progression of LGMD can be mild to severe, with the most severe forms associated with dramatic muscular weakness that reduces life expectancy. LGMD is classified into two main groups: autosomal dominant LGMD type 1 (LGMD1) and autosomal recessive LGMD type 2 (LGMD2), the latter comprising over 16 types (A–Q) (Nigro and Savarese 2014). LGMD2 is more common than LGMD1, but its incidence varies in different countries depending on the degree of consanguinity and allele carriage (Guglieri, *et al.* 2008a, Lo, *et al.* 2008). Therefore, the geographical and ethnic origins of suspected LGMD patients need to be considered when formulating the differential diagnosis. LGMD type 2D (LGMD2D, OMIM 608099) is caused by mutations in the alpha-sarcoglycan gene (*SGCA*, OMIM 600119) located on chromosome 17q21, which is 10kb in length with 10 exons. Alpha-sarcoglycan (originally called adhalin) is a 50kDa, 387 amino acid protein that is essential for the mechanical

stability of muscle fiber membranes and membrane integrity during muscle contraction.

Since there are no pathognomonic features of LGMD2D, diagnosis relies on a combination of immune analysis (immunohistochemistry (IHC) and western blotting) and mutation screening (Louhichi *et al.* 2004). However, immune-based analyses are not specific and it is important to identify specific mutations in a given population to correctly characterize the disease subtype. Here we present the clinical and molecular genetics of a native consanguineous Saudi family with autosomal recessive LGMD2D to highlight how the molecular diagnosis is relevant to patient and family management.

CLINICAL SUMMARY

A seven-year-old Saudi daughter of consanguineous parents presented with a history of progressive proximal muscle weakness and recurrent falls, toe walking, and difficulty raising her arms above her head. Proximal muscle weakness was first noted in the lower limbs, which subsequently spread to the upper limbs. There were signs of calf pseudohypertrophy, mild scapular winging, and hyperlordosis. The mother had not experienced any complications during pregnancy and, on antenatal examination, there had been no signs of muscle disorders.

The patient's pulmonary function tests were normal. Creatinine phosphokinase (CPK) levels ranged between 7 000 and 25 000 units/L (normal <215 u/L) throughout the clinical course, and myopathic changes were evident on electromyography. There was no known family history of neuromuscular disorders, but the family history was significant for consanguinity, her parents being first cousins of Saudi origin. Furthermore, both her father's and mother's grandparents were first cousins of Saudi origin (Figure 1). There was a family history of hypertension in the paternal line and a maternal aunt with an undiagnosed disorder characterized by muscle cramping that required a walking stick to facilitate ambulation and premature death at 49 years of age.

Since muscular dystrophy was suspected, biopsy samples were obtained from the patient for histopathological examination by commercial laboratory services (Unilabs, London, UK). Fresh blood was collected in lithium-heparin tubes from the patient for conventional cytogenetic studies and blood samples were collected from the patient, parents, and three other siblings in EDTA tubes for DNA extraction.

Genomic DNA was extracted from 4 ml whole blood using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol and quantified by spectrophotometry (MaestroNano, Las Vegas, NV, USA). Samples were stored at –20 °C until use. *SGCA* exons and a flanking short intron were sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit and run on the 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequencing results were analyzed using SeqScape v3.0 software (Applied Biosystems) and compared to Ensemble gene reference number ENSG00000108823. Primer sequences and PCR conditions are available on request. Conventional cytogenetic analysis and multiplex ligation-dependent probe amplification (MPLA) analysis to detect any large gene deletions or duplications were performed by commercial laboratory services (Unilabs, Lausanne, Switzerland). *In silico* analysis was performed using several different tools to confirm any functional effects: PolyPhen–2 (http://genetics.bwh.harvard.edu/pph2), Mutation Taster (http://www.mutationtaster.org), and SIFT (http://sift.bii.a-star.edu.sg). Multiple protein sequence alignment programs were used to check for evolutionary conservation of the amino acid sequence.

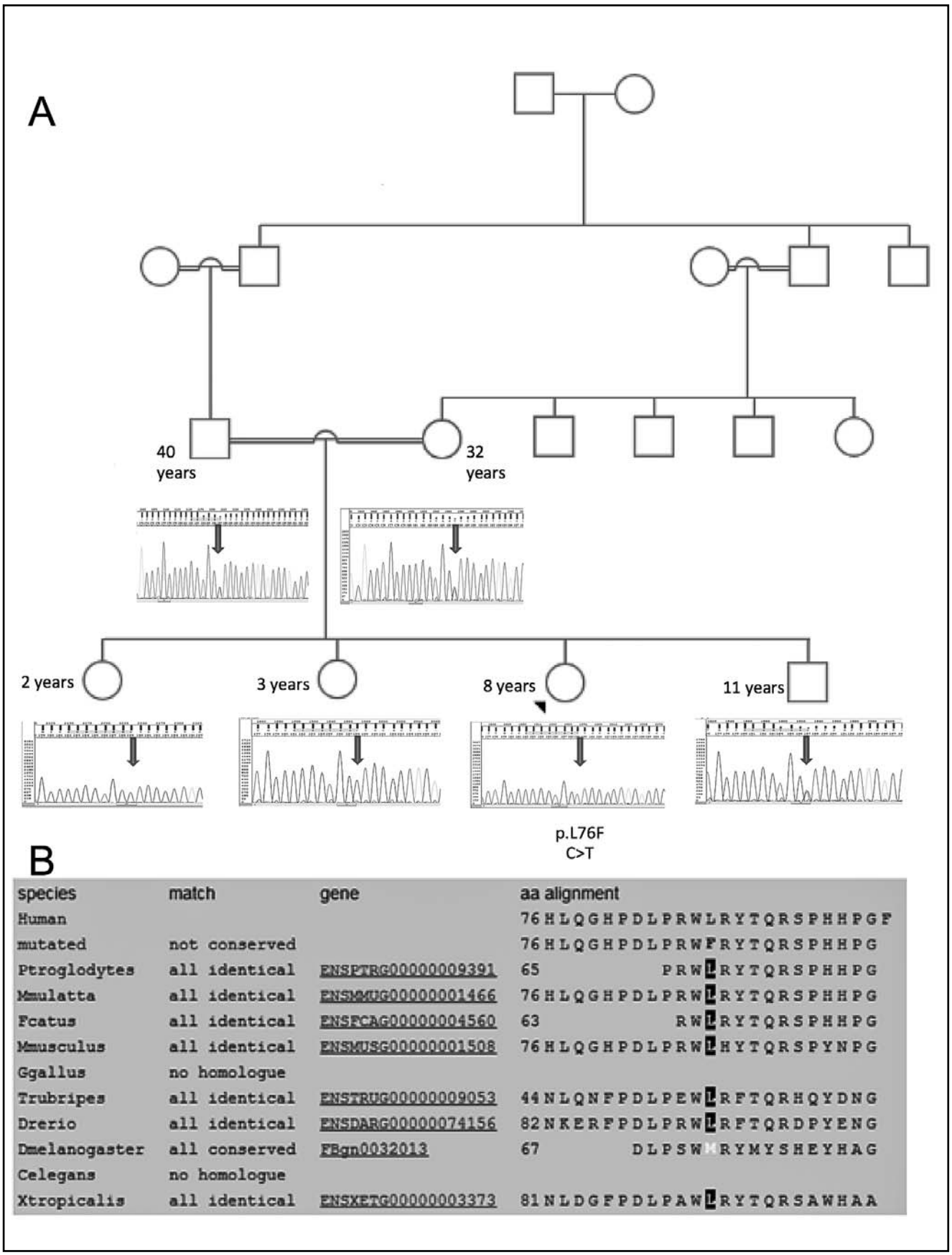
The parents and family members provided written informed consent for molecular studies and for the publication of this case report. The study conformed to the principles of the Declaration of Helsinki, and the Maternity & Children Hospital ethics committee approved the study protocol,

PATHOLOGICAL FINDINGS

Histopathological examination of the muscle biopsy revealed a few muscle fibers of increased diameter, an increase in the endomysial connective tissue, and a few necrotic muscle fibers with phagocytosis characteristic of muscular dystrophy. Examination of immunohistochemically-stained tissue sections showed a significant reduction in sarcoglycan expression, particularly alpha-sarcoglycan, with normal dystrophin and spectrin staining patterns. Unfortunately, histological illustrations cannot be shown as all pathology information are based on a written report from the commercial lab (Unilabs, London, UK).

Since sarcoglycans, especially alpha-sarcoglycan, were low or absent, SGCA gene mutations were first examined as recommended (Babameto-Laku, *et al.* 2011). Sequence analysis of *SGCA* in the index case revealed a homozygous mutation (c.226 C>T (p.L76F)) in exon 3. This substitution results in a leucine to phenylalanine amino acid change (Figure 1). The parents and one sibling were heterozygous for this mutation, while two other siblings were homozygous for the wild type allele (Figure 1). The mutation was not detected in 80 ethnically-matched controls from the same geographic area. Based on the written report from the commercial lab (Unilabs, Lausanne, Switzerland), no large deletions or duplications were detected by MPLA analysis, and conventional cytogenetic analysis showed a normal female karyotype (46,XY).

In silico analysis of the p.L76F mutation showed that this amino acid change would be expected to alter protein structure, with a SIFT score of 0.03, PolyPhen score of 1, and Mutation Taster confirming the deleterious effect of this amino acid substitution on protein func-



species	match	gene	aa alignment
Human			76 H L Q G H P D L P R W L R Y T Q R S P H H P G F
mutated	not conserved		76 H L Q G H P D L P R W F R Y T Q R S P H H P G
Ptroglodytes	all identical	ENSPTRG00000009391	65 P R W L R Y T Q R S P H H P G
Mmulatta	all identical	ENSMMUG00000001466	76 H L Q G H P D L P R W L R Y T Q R S P H H P G
Fcatus	all identical	ENSFCAG00000004560	63 R W L R Y T Q R S P H H P G
Mmusculus	all identical	ENSMUSG00000001508	76 H L Q G H P D L P R W L H Y T Q R S P Y N P G
Ggallus	no homologue		
Trubripes	all identical	ENSTRUG00000009053	44 N L Q N F P D L P E W L R F T Q R H Q Y D N G
Drerio	all identical	ENSDARG00000074156	82 N K E R F P D L P R W L R F T Q R D P Y E N G
Dmelanogaster	all conserved	FBgn0032013	67 D L P S W H R Y M Y S H E Y H A G
Celegans	no homologue		
Xtropicalis	all identical	ENSXETG00000003373	81 N L D G F P D L P A W L R Y T Q R S A W H A A

Fig. 1. A. Pedigree of the Saudi family with LGMD2D. Double lines are indicative of consanguineous union. Arrow indicates affected individual. DNA sequences were available for the affected family. The proband is homozygouse for the mutation. Father, mother and a sun are heterozygouse for the mutation, while two daughters are homozygouse for the wild type. **B**. Alignment of the SGCA protein sequences across species. The altered amino acid of c.226 C>T (p.L76 F) is highly evolutionarily conserved among the various species.

tion. Amino acid sequence alignment of the SGCA protein across different species revealed that the position 76 leucine is evolutionarily conserved across different species (Figure 1).

DISCUSSION

The American Academy of Neurology and the American Association of Neuromuscular & Electrodiagnostic Medicine recently issued new guidelines on the diagnosis and management of LGMD patients that emphasized the need to identify muscular dystrophy subtypes by genetic testing (Kang *et al.* 2015). The goal of gene-specific LGMD diagnosis is to direct individualized patient management, especially with respect to pulmonary and cardiac complications. The phenotypic variability of many diseases is often associated with genetic heterogeneity, particularly the sarcoglycanopathies (Harel *et al.* 2004; Fanin *et al.* 2014). Moreover, the frequency of sarcoglycanopathy types and mutations varies between populations (Guglieri *et al.* 2008b). Therefore, identifying the specific mutation affecting different populations is important for diagnosis and management of both the patient and family members.

Here, the patient had a homozygous mutation in exon 3 of *SGCA*. Her parents were heterozygous carriers of the mutation and, of her three siblings, one was a carrier and two were unaffected. This information was useful for family members, especially for future healthcare of sibling who are carriers for the mutation. This is the first report of an alpha-sarcoglycanopathy harboring the p.L76F mutation in a Saudi Arabian patient and, to our knowledge, the p.L76F mutation has only previously been reported in one other Turkish family with LGMD2D (Diniz, *et al.* 2014). Boyden *et al.* previously described thirteen consanguineous Saudi Arabian families with LGMD2 (Boyden, *et al.* 2010), of whom three families were diagnosed with LGMD2D, albeit carrying different *SGCA* gene mutations: two with the known R34H mutation (c.101G>A p.R34H) and a novel splice site mutation (c.584+5G>A) (Boyden, *et al.* 2010). However, none of these families carried the p.L76F mutation.

Over 70 deletion, insertion, substitution, and duplication mutations have been reported in *SGCA* (Diniz, *et al.* 2014). The most commonly observed mutation responsible for the LGMD2D subtype is the 229CGC>TGC (R77C) mutation in exon 3 of *SGCA* (Nigro and Savarese 2014). This mutation has not been reported in Saudi patients, suggesting that LGMD2D in Saudi Arabia may have a different genetic etiology.

The functional consequences of *SGCA* mutations are not well characterized. The most common mutation, p.R77C, causes misfolding of the protein that results in sequestration of the protein in the endoplasmic reticulum and complete loss of SGCA protein expression in muscle biopsies (Sandonà and Betto 2009). Similarly, we observed significant reduction of SGCA in our case, similar to the findings of the p.L76F mutation case from Turkey (Diniz *et al.* 2014). The p.L76F mutation may, therefore, result in similar consequences as the p.R77C mutation by impairing SGCA protein trafficking. *In silico* analysis using three different algorithms (PolyPhin, SIFT, and Mutation Taster) showed that the p.L76F amino acid change was deleterious and damaging to protein levels. Interestingly, the leucine at position 76 is evolutionarily conserved across different species, suggesting that this amino acid may be critical to protein function. Moreover, the absence of p.L76F in 80 healthy controls from the same ethnic background and co-segregation of the mutation with the clinical phenotype suggests that p.L76F might be causative for the LGDM2D phenotype seen here. However, a full functional analysis of the p.L76F mutation is required to confirm this association.

In summary, this is the second report of LGMD2D putatively caused by the p.L76F mutation. The appropriate molecular analysis – in this case sequencing – allowed appropriate patient management and the provision of genetic counseling to the family members to enable them to make informed decisions. Sarcoglycanopathies and their causative mutations vary between populations. If an alpha-sarcoglycanopathy is suspected, *SGCA* gene sequencing is an appropriate and cost-effective first step that avoids the need for screening all known causative genes. However, modern sequencing techniques, such as next generation sequencing, are already becoming more cost effective for the molecular genetics diagnosis of sarcoglycanopathies, and knowledge of population-specific mutations will be useful for designing targeted NGS panels for clinical diagnostics.

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REFERENCES

1 Babameto-Laku A, Tabaku M, Tashko V, Cikuli M, Mokini V (2011). The first case of primary alpha-sarcoglycanopathy identified in Albania, in two siblings with homozygous alpha-sarcoglycan mutation. Genet Couns. **22**(4): 377–383.

2 Boyden SE, Salih MA, Duncan AR, White AJ, Estrella EA, Burgess SL, Seidahmed MZ, Al-Jarallah AS, Alkhalidi HM, Al-Maneea WM, Bennett RR, Alshemmari SH, Kunkel LM, Kang PB (2010). Efficient identification of novel mutations in patients with limb girdle muscular dystrophy. Neurogenetics. **11**(4): 449–455.
3 Diniz G, Tosun Yildirim H, Akinci G, Hazan F, Ozturk A, Yararbas K, Tukun A (2014). Sarcolemmal alpha and gamma sarcoglycan protein deficiencies in Turkish siblings with a novel missense mutation in the alpha sarcoglycan gene. Pediatr Neurol. **50**(6): 640–647.
4 Fanin M, Nascimbeni AC, Angelini C (2014). Gender difference in limb-girdle muscular dystrophy: a muscle fiber morphometric study in 101 patients. Clin Neuropathol. **33**(3): 179–85.
5 Guglieri M, Magri F, D'Angelo MG, Prelle A, Morandi L, Rodolico C, *et al.* (2008a). Clinical, molecular, and protein correlations in a large sample of genetically diagnosed Italian limb girdle muscular dystrophy patients. Hum Mutat. **29**(2): 258–266.
6 Guglieri M, Straub V, Bushby K, Lochmüller H. Limb-girdle muscular dystrophies (2008b). Curr Opin Neurol. **21**(5): 576–84.
7 Harel T, Goldberg Y, Shalev SA, Chervinski I, Ofir R, Birk OS (2004). Limb-girdle muscular dystrophy 2I: phenotypic variability within a large consanguineous Bedouin family associated with a novel FKRP mutation. Eur J Hum Genet. **12**(1): 38–43.
8 Kang PB, Morrison L, Iannaccone ST, Graham RJ, Bönnemann CG, Rutkowski A, *et al.* (2015). Evidence-based guideline summary: evaluation, diagnosis, and management of congenital muscular dystrophy: Report of the Guideline Development Subcommittee of the American Academy of Neurology and the Practice Issues Review Panel of the American Association of Neuromuscular & Electrodiagnostic Medicine. Neurology. **84**(13): 1369–1378.
9 Lo HP, Cooper ST, Evesson FJ, Seto JT, Chiotis M, Tay V, Compton AG, Cairns AG, Corbett A, MacArthur DG, Yang N, Reardon K, North KN (2008). Limb-girdle muscular dystrophy: diagnostic evaluation, frequency and clues to pathogenesis. Neuromuscul Disord. **18**(1): 34–44.
10 Louhichi N, Triki C, Meziou M, Rouis S, Ayadi H, Fakhfakh F (2004). Contribution of immunological and genetic investigations to improve classification of patients with congenital muscular dystrophy. Neurosciences (Riyadh). **9**(4): 247–53.
11 Nigro V, Savarese M (2014). Genetic basis of limb-girdle muscular dystrophies: the 2014 update. Acta Myol. **33**(1): 1–12. Review.
12 Sandonà D, Betto R (2009). Sarcoglycanopathies: molecular pathogenesis and therapeutic prospects. Expert Rev Mol Med. **11**: e28.