Folia Neuropathologica

Case report of an adolescent girl with limb-girdle muscular dystrophy type 2B – the usefulness of muscle protein immunostaining in the diagnosis of dysferlinopathies

Sylwia Szymanska[1], Dariusz Rokicki[2], Agnieszka Karkucinska-Wieckowska[1], Tamara Szymanska-Debinska[1], Elzbieta Ciara[3], Rafal Ploski[4], Wieslawa Grajkowska[1,5], Maciej Pronicki[1]

[1]Department of Pathology, The Children's Memorial Health Institute, Warsaw, [2]Department of Pediatrics, Nutrition and Metabolic Disorders, The Children's Memorial Health Institute, Warsaw, [3]Department of Medical Genetics, The Children's Memorial Health Institute, Warsaw, [4]Department of Medical Genetics, Centre of Biostructure, Medical University of Warsaw, [5]Department of Clinical and Experimental Neuropathology, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

Folia Neuropathol 2014; 52 (4): 452-456 DOI: 10.5114/fn.2014.47847

Abstract

Dysferlinopathies are rare disorders of muscle that present two main phenotypes: Miyoshi myopathy with primarily distal weakness and limb-girdle muscular dystrophy type 2B (LGMD2B) with primarily proximal weakness. They are caused by mutations in the gene encoding the skeletal muscle protein dysferlin, which is involved in muscle repair. The clinical presentation of the disease is rather uncharacteristic, and molecular genetic testing is long-lasting; thus muscle biopsy may be essential in the diagnostic process. Histology itself reveals non-specific changes, but a variety of currently available muscle protein immunostains may be very helpful. We present a 19-year-old girl with epilepsy and elevated creatine phosphokinase (CPK) concentration. Due to increased CPK, myopathy was suspected and muscle biopsy was performed. Light microscopy showed no distinctive myopathic changes, and electron microscopy showed no abnormalities. Extended immunohistochemistry, performed much later, showed complete absence of dysferlin immunostaining. Based on that result, the diagnosis of LGMD2B was made, with subsequent genetic testing to be done. Two known pathogenic variants were found in the DYSF gene, confirming the diagnosis of LGMD2B and allowing proper genetic counseling.

Key words: *dysferlinopathies, limb-girdle muscular dystrophy type 2B (LGMD2B), dysferlin, immunohistochemistry, muscle, DYSF gene.*

Introduction

Limb-girdle muscular dystrophy type 2B (LGMD2B) is an autosomal recessive phenotype of dysferlinopathies, muscle disorders caused by mutations in the dysferlin gene (*DYSF*) [9]. The *DYSF* gene is located on chromosome 2p12-14 and encompasses 55 exons spanning over 150 kb of genomic DNA. It encodes a 230-kDa type-II transmembrane protein, dysferlin, which contains a large intracellular cytoplasmic *N*-terminal domain, an extreme *C*-terminal transmembrane domain, and a short *C*-terminal extracel-

Communicating author:
Maciej Pronicki, Department of Pathology, The Children's Memorial Health Institute, Al. Dzieci Polskich 20, 04-730 Warsaw, Poland, phone: +48 228151960, fax: +48 228151975, e-mail: m.pronicki@czd.pl

lular domain. Its expression is widespread in different tissues, especially in skeletal muscles and cardiac muscle [2]. In skeletal muscle, dysferlin is located at the plasma membrane, as well as in cytoplasmic vesicles [3]. Its role is associated with muscle repair. In human patients with LGMD2B, dysferlin expression is absent. Clinically, it is characterized by weakness in the proximal muscles at onset, involving predominantly the lower limbs. At late stages of the disease, loss of muscle bulk in the pelvic girdle and calf may appear. This may result in frequent falls, and difficulty in climbing stairs, rising from the floor, and running. With the progression of the condition, patients may have problems with walking [1,4]. Onset is typically in the late teens or early adulthood, with no previous history of muscle weakness. Laboratory tests show increased levels of the muscle enzyme creatine kinase, typically 10 to 150 times above normal levels. Cardiac muscle and respiratory muscles are not involved in this disorder [9]. Symptoms are not specific enough; thus LGMD2B should be confirmed by identifying a defect either in the *DYSF* gene, which is done on a DNA sample from the blood, or by Western blot analysis of skeletal muscle biopsy. It was proved [5] that the reduction of dysferlin expression to 20% of normal values in skeletal muscle or in peripheral blood monocytes is associated with 100% coexistence of pathogenic mutations in the *DYSF* gene; however, long *DYSF* gene sequencing is time-consuming. Therefore, microscopic analysis of muscle biopsy may be essential in the diagnostic process. Histology itself reveals non-specific changes such as centrally located nuclei, fiber splitting, scattered necrotic and regenerating fibers, and an occasional perivascular infiltrate comprising lymphocytes and macrophages, sometimes leading to a misdiagnosis of polymyositis. There is increased connective tissue in the endomysium and perimysium. Fiber type distribution is normal and rimmed vacuoles and ragged red fibers are absent [6,8,13,14]. These microscopic findings may also be seen in other limb-girdle muscular dystrophies, caveolinopathies and dystrophinopathies; thus immunohistochemistry seems to be essentially helpful. In healthy muscle, dysferlin immunoreactivity is localized along the plasma membrane of muscle fibers. The pathological pattern of immunostaining is either complete absence of or marked reduction in LGMD2B, depending on the nature of the genetic mutation [11]. We present a 19-year-old girl with epilepsy and elevated creatine phosphokinase (CPK) concentration. Due to increased CPK a myopathy was suspected and muscle biopsy was performed. Light microscopy showed no distinctive myopathic changes, and electron microscopy showed no abnormalities. Extended immunohistochemistry performed much later within our research project showed complete absence of dysferlin immunostaining. The diagnosis of LGMD2B was then made.

Case report

We present a case report of a 19-year-old girl who was suspected of myopathy at the age of 10. She was born as a second child of her parents; pregnancy and delivery were not complicated. The period of infancy was uneventful. Psychomotor development was normal. At the age of one, she presented an episode of convulsions that happened again when she was two and a half. The epilepsy was diagnosed with consecutive introduction of treatment (valproic acid). Epilepsy attacks subsided but increased levels of transaminases (up to 170 U/l) occurred with time. The medication was changed without a satisfactory effect on hypertransaminasemia. In 2004 the patient was admitted to The Children's Memorial Health Institute because of a persistent increase in transaminase levels. Laboratory tests performed at the time showed increased transaminases (AlAT 163 U/l; AspAT 96 U/l) and elevated CPK concentration (2402 U/l). Because of elevated CPK, she has been suspected of having a myopathic disorder since then. In 2005, during her follow-up visit to the Outpatient Metabolic Clinic, she complained of occasional muscle pain. Otherwise, she was in good general condition. Physical examination did not reveal muscle weakness, atrophy or hypertrophy of muscles, and muscle tension was correct. Cardiological consultation did not reveal cardiomyopathy. Electromyography (EMG) was also performed, but the result was within the normal range. The most sensitive and specific parameter for myopathy in conventional EMG, which is decreased duration of motor unit potentials (MUP), was not observed. Muscle biopsy was planned but had to be postponed due to the patient's infection. One year later, the girl was admitted to the hospital again to undergo muscle biopsy. She remained in good condition; levels of transaminases were lower, but constantly increased (AlAT 107 U/l; AspAT 72 U/l); CPK > 3000 U/l. Histological findings in the performed muscle biopsy were consistent with moderate myo-

pathic changes including an increased number of centrally located, single necrotic fibers, and unequally intensive NADH-D reaction. There were no features of regeneration or endomysial fibrosis. Findings characteristic of neurogenic disorders were not present. In electron microscopy there were no diagnostic ultrastructural changes. Spectrophotometric analysis of mitochondrial respiratory chain complex enzyme activities revealed decreased complex IV activity (6.6%; normal levels 19-33.8%) and complex I activity (6.8%; normal levels 8.2-18%). Frozen samples of muscle were protected for further examination. The patient was discharged from hospital with suspicion of undefined myopathy. In 2008, additional immunostainings were performed from frozen samples (merosin, adhalin and dystrophin [DYS.1, DYS.2, DYS.3]), but expression was normal. Recently, we decided to assess her muscle biopsy once again within a research project, using an extended panel of muscle protein immunostains. This time, second-look examination revealed a pattern of complete absence of dysferlin immunostaining. In view of the clinical presentation and histologic findings, the diagnosis of LGMD2B was made (Figs. 1 and 2). Whole exome sequencing (WES) was conducted to identify the molecular basis of the disease. Whole exome sequencing was performed on a HiSeq 1500 using an Exome Enrichment Kit (Illumina) [12]. Generated reads were aligned to the hg19 reference human genome. Alignments were viewed with Integrative Genomics Viewer v.2.2.34.

Two known pathogenic variants in the *DYSF* gene were revealed: missense mutation c.6124C>T in exon 54 and splice-site mutation c.1180+5G>A in intron 12 (Fig. 3). The missense change is associated with severe reduction of dysferlin activity, and the intronic variant is predicted to create a cryptic splice donor site, but its protein effect is unknown yet. This has to be further validated at the transcriptional level. Mutation numbering is based on the cDNA sequence (human *DYSF*, GenBank NM_003494.3) according to journal guidelines (www.hgvs.org/mutnomen).

Discussion

LGMD2B is a rare autosomal recessive muscle disorder caused by mutation in the *DYSF* gene, mostly missense or null alleles [10]. Onset of the disease is usually in the proximal lower-limb musculature in the late teens or later, most commonly between 20 and 30 years of age. Progress is rather slow [4]. Our patient presented the first symptoms when she was 10 years old, which was relatively early; thus suspicion of LGMD2B was not obvious. Recently, Fanin *et al*. [7] found that males with LGMD may be clinically more severely affected than females, although the mechanism remains elusive. Our patient has always remained in good general condition, and sporadic muscle pain has been her only complaint while CPK has been constantly and significantly increased (> 3000 U/l). Massive elevation of serum CK concentration is thought [4,13] to be one of the typical LGMD2B symptoms, but it is not a specific marker – it

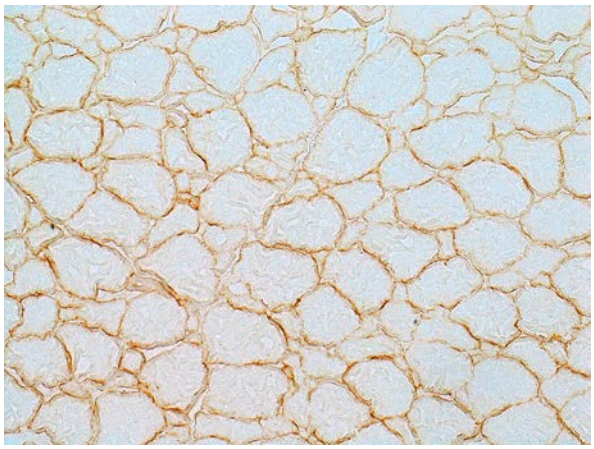

Fig. 1. Image of skeletal muscle dysferlin IHC control positive reaction. Original magnification × 400.

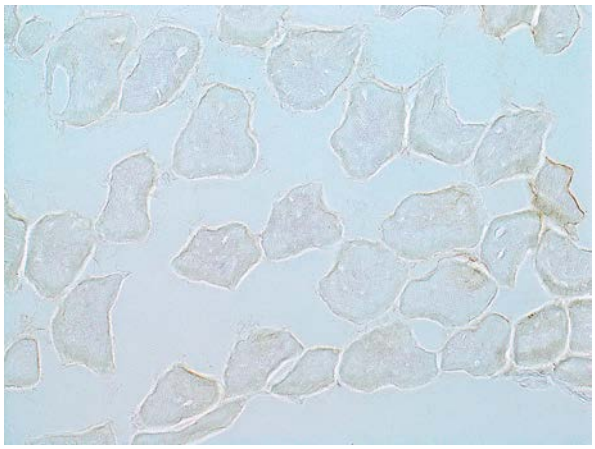

Fig. 2. Image of skeletal muscle dysferlin IHC negative reaction in our patient's muscle biopsy. Original magnification × 400.

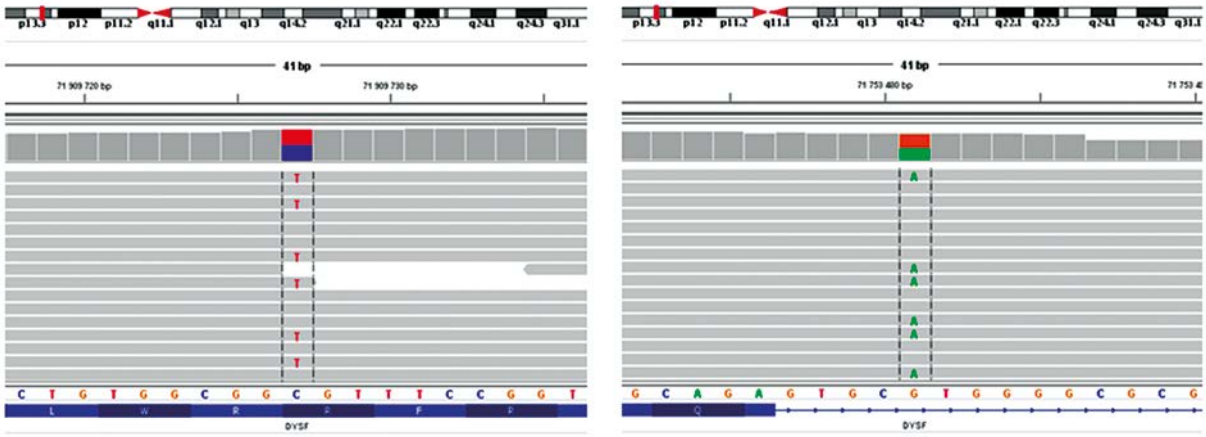

Fig. 3. Integrative Genomics Viewer view of DYSF mutations: c.6124C>T (left) and c.1180+5G>A (right) in the patient by whole-exome sequencing.

only indicates muscle damage. One method that leads to a firm LGMD2B diagnosis is molecular genetic testing. Muscle biopsy Western immunoblotting almost always indicates a primary dysferlinopathy. *DYSF* is the only gene known to be associated with dysferlinopathy [1]. There were three testing possibilities – targeted mutation analysis focused on the two most common mutations among Jews [1] (1624delG, and 927delG); *DYSF* gene sequencing [1]; and mutation scanning that detects sequence variants but only in 80% of individuals [15]. Although genetic tests are the most precise methods and they must be done to confirm the LGMD2B diagnosis, they are time-consuming and expensive, and are often done following a clue from the muscle biopsy or examination. As histologic light microscopic features are non-characteristic, immunostaining is essential. Our patient was first suspected of having a muscle disorder almost 10 years ago when immunohistochemistry was limited, and thus the exact diagnosis was established only recently, after performing additional staining within our research project. LGMD2B is genetically transferred, but the risk that offspring will inherit one faulty copy of the dysferlin gene and therefore will all be carriers but unaffected is unlikely. Therefore, carrier testing is not necessary unless the risks are increased due to intra-familial marriage. We present this case as an example of the practical usefulness of immunostaining in the diagnosis of LGMD2B. We believe that an extended immunohistochemical panel of muscle proteins should be examined in all patients with non-specific histologic features of myopathy to increase the detection rate of known limb girdle muscle dystrophies.

An alternative diagnostic approach in the described patient was provided by fast and cost-effective next generation sequencing (NGS). This method, simultaneously identifying mutations affecting both the most frequent and rare genes, will greatly improve the precise diagnosis of heterogeneous limb-girdle muscular dystrophies, especially for patients with mild, non-specific or atypical phenotype.

Acknowledgments

The work was supported by grants of The Children's Memorial Health Institute for young researchers, no. M4/2012 and 136/2013, Polish National Science Centre (NCN) grant 2011/01/B/NZ4/03455, and EU Structural Funds, project POIG.02.01.00-14-059/09.

References

1. Aoki M. Dysferlinopathy. In: Pagon RA, Adam MP, Ardinger HH, Bird TD, Dolan CR, Fong CT, Smith RJH, Stephens K (eds.). GeneReviews® University of Washington, Seattle 2004.
2. Bansal D, Campbell KP. Dysferlin and the plasma membrane repair in muscular dystrophy. Trends Cell Biol 2004; 4: 206-213.
3. Bansal D, Miyake K, Vogel SS, Groh S, Chen CC, Williamson R, McNeil PL, Campbell KP. Defective membrane repair in dysferlin-deficient muscular dystrophy. Nature 2003; 423: 168-172.
4. Bushby KMD, Straub V, Lochmuller H, Eagle M, Guglier M, Hastings L. National Commissioning Group (NCG) and the Clinical team at Newcastle, LGMD 2B – Muscular Dystrophy Campaign. www.muscular-dystrophy.org
5. Cacciottolo M, Numitone G, Aurino S, Caserta IR, Fanin M, Politano L, Minetti C, Ricci E, Piluso G, Angelini C, Nigro V. Muscular dystrophy with marked dysferlin deficiency is consistently caused by primary dysferlin gene mutations. Europ J Hum Genet 2011; 19: 974-980.

6. Fanin M, Angelini C. Muscle pathology in dysferlin deficiency. Neuropathol Appl Neurobiol 2002; 28: 461-470.
7. Fanin M, Nascimbeni AC, Angelini C. Gender difference in limb-girdle muscular dystrophy: a muscle fiber morphometric study in 101 patients. Clin Neuropathol 2014; 33: 179-185.
8. Gallardo E, Rojas-Garcia R, de Luna N, Pou A, Brown RH, Illa I. Inflammation in dysferlin myopathy: immunohistochemical characterization of 13 patients. Neurology 2001; 57: 2136-2138.
9. Karpati G. Structural and Molecular Basis of Skeletal Muscle Diseases. Diseases associated with sarcolemmal and extracellular matrix defects: Dysferlinopathies. ISN Neuropath Press, Basel 2002; pp. 29-39.
10. Nigro V, Savarese M. Genetic basis of limb-girdle muscular dystrophies: the 2014 update. Acta Myol 2014; 33: 1-12.
11. Piccolo F, Moore SA, Ford GC, Campbell KP. Intracellular accumulation and reduced sarcolemmal expression of dysferlin in limb-girdle muscular dystrophies. Ann Neurol 2000; 48: 902-912.
12. Ploski R, Pollak A, Müller S, Franaszczyk M, Michalak E, Kosinska J, Stawinski P, Spiewak M, Seggewiss H, Bilinska ZT. Does p.Q247X in TRIM63 cause human hypertrophic cardiomyopathy? Circ Res 2014; 114: e2-5.
13. Prelle A, Sciacco M, Tancredi L, Fagiolari G, Comi GP, Ciscato P, Serafini M, Fortunato F, Zecca C, Gallanti A, Chiveri L, Bresolin N, Scarlato G, Moggio M. Clinical, morphological and immunological evaluation of six patients with dysferlin deficiency. Acta Neuropathol (Berl) 2003; 105: 537-542.
14. Serratrice G, Pellissier JF, N'Guyen V, Attarian S, Pouget J. Dysferlinopathy. Example of a new myopathy. Bull Acad Natl Med 2002; 186: 1025-1032.
15. Takahashi T, Aoki M, Tateyama M. Mutational and clinical features of Japanese patients with dysferlinopathy. Neurology 2003; 60 Suppl: A233.