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

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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 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-
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 normal values in skeletal muscle or in peripheral tissues, especially in skeletal muscles and cardiac muscles. Its role is associated with muscle repair. In human patients with LGMD2B, dysferlin expression is absent. The pathological pattern of immunostaining 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. 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 (AIAT 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 (AIAT 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).

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

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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.
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.

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