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Diagnosis by protein analysis of dysferlinopathy in two patients mistaken as polymyositis

Corrado Angelini[1], Wolfgang Grisold[2], Vincenzo Nigro[3]

[1] Department of Neurosciences, University of Padova, Italy; [2] Kaiser Franz Joseph Hospital, Department of Neurology, Wien, Austria; [3] Telethon Institute of Genetics Medicine, TIGEM, Naples, Italy

We investigated the clinical and molecular pattern of two young men affected by dysferlinopathy, that was first diagnosed as polymyositis. We show that their symptoms and clinical course although progressive were peculiar, as well as their biopsy suggesting a subsequent analysis of dysferlin protein by western blotting. Molecular analysis of dysferlin gene revealed pathogenetic mutations in both cases.

In such cases a screening with Western blot followed by DNA analysis of dysferlin gene is therefore recommended. We present a diagnostic algorythm for patients with suspected myositis but presenting signs of disease progression and poor response to steroids.

Key words: Dysferlin, LGMD2B, Western blot

Introduction

LGMD2B or dysferlinopathy is an underdiagnosed clinical entity and both its identification and its treatment approach are relevant for clinicians and geneticists. Muscle biopsy samples from patients with dysferlin deficiency might be inflammatory (1) and ultrastructural examination shows numerous structural membrane defects (2), attributed to the fact that dysferlin deficient fibers fail to reseal plasmalemmal lesions occurring mainly during eccentric muscle contractions. We present two LGMD2B cases where an abrupt onset of weakness and markedly elevated CK levels and myalgia lead to a mistaken diagnosis of polymyositis and subsequent prolonged and inappropriate immunosuppressive therapy. Molecular and genetic studies are needed to obtain a definite diagnosis among cases with apparent refractory myositis or unexplained proximal muscular weakness with high CK, pain, myalgia and muscle swelling.

Case report

Patient 1

During a field trip in Persia in 2006 we studied numerous neuromuscular cases, and we investigated a 36 year-old man that had onset of disease and muscle weakness at age 18, and became rapidly wheelchair-bound for a proximo-distal myopathy since 22 years. Since his disease onset coincided with Iran-Iraq war and he presented muscle pain and fatigability with a high CK levels (5300 U/L), a toxic polymyositis was suspected. The patient was treated with steroids without benefit. The nature of the presumed toxic agents used during the Iran-Iraq war was not further defined. An EMG showed a myopathic pattern and short motor unit. A vastus lateralis open muscle biopsy showed numerous lobulated fibers, type 1 fibres prevalence, degenerating fibers and macrophagic reaction (Fig. 1). We performed a detailed molecular analysis. Dysferlin protein analysis by western blotting showed absent protein. The screening of mutations in the dysferlin gene resulted in the identification of two compound heterozygous mutations: one novel missense mutation in exon 38 (c.4024C > T, p.R1342W) and one single-base duplication in exon 26 causing a frame-shifting (c.2706dupC, p.K903QfsX4). The effect of the novel missense mutation found was predicted to be "probably damaging" using Polyphen prediction software (http://genetics.bwh.harvard.edu).

Patient 2

A 14 year-old boy presented burning sensation in his legs and during a soccer game suddenly fell forward. During the following months his legs were sore and his thighs

Address for correspondence: Corrado Angelini, MD, Department of Neurosciences, University of Padova, via Giustiniani 5, 35128 Padova, Italy. E-mail: corrado.angelini@unipd.it

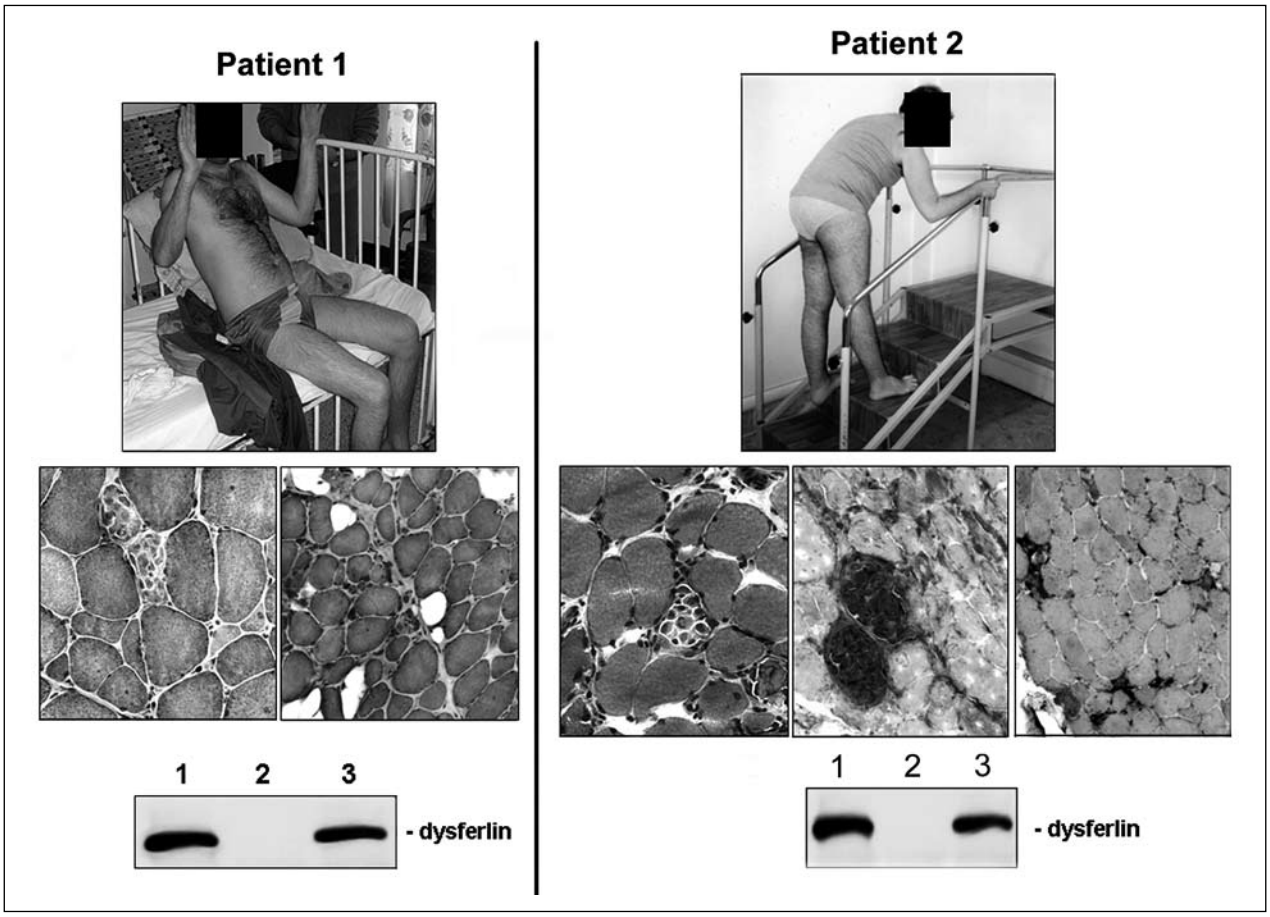

Figure 1. Patient 1 (panel on the left) shows atrophic thighs and calves and difficulty raising arms. Muscle biopsy histopathology shows fibres undergoing degeneration and fibro-fatty replacement with trichrome stain. Dysferlin western blot shows absent protein (lane 2). Patient 2 (panel on the right) climbs stairs with rail support and presents straight leg, bent spine and Cushingoid appearance. First muscle biopsy shows some degenerating fibres with haematoxylin-eosin stain, which are undergoing phagocytosis with acid phosphatase stain. Note lower extent of phagocytosis in the second biopsy. Dysferlin western blot analysis shows absent dysferlin protein (lane 2).

were swollen. CK was 22,000 U/L. He was hospitalized 6 months later in a pediatric rheumathology unit.

A muscle biopsy showed diffuse macrophage infiltration, lymphocytic reaction, degenerative and regenerating muscle fibers, increased expression of MHC class I molecules. Muscle protein analysis by western blot showed normal dystrophin and alpha-sarcoglycan. These changes were interpreted as myositis.

A treatment with Prednisone 50 mg/day for 15 months without response was performed. Thereafter, a treatment with azathioprine and cyclosporine were tried without response, IVIg were also added without clinical response and he was kept on steroids.

After 15 months of prednisone he developed a Cushingoid appearance, buffalo hump, massive weight gain and osteoporosis.

Neurological examination showed waddling gait, difficulty raising from the floor, proximal muscle weakness (ileopsoas muscle 4/5, gluteus muscle 4/5, tibialis anterior muscle 4/5). EMG showed small polyphasic potentials. Muscle CT scan showed diffuse abnormality of proximal muscle in lower extremities. At age 17 years a second muscle biopsy on vastus lateralis muscle showed few inflammatory cells that appeared secondary to necrosis as demonstrated by the predominance of macrophages in the infiltrate (Fig. 1). Dysferlin protein analysis by western blot showed absent dysferlin protein. The patient was therefore investigated for dysferlin gene mutations and two compound heterozygote mutations were identified: one frame-shifting deletion in exon 23 (c.2200_2205delinsT, p.T734SfsX17) and one frame-shifting deletion in exon 32 (c.3516_3517del, p.S1173X). This patient was therefore a compound heterozygote of a null mutation and a missense point mutation but the protein resulted absent.

When the LGMD2B diagnosis was established, steroid treatment was tapered down and patient benefited from weight loss and less fluid retention. He had to be supplemented with Ca^{++} and biphosphonate for abnormal bone densitometry, due to the long term steroid treatment. His CK levels decreased from 12,752 U/L to 7,785 U/L.

Discussion

A false diagnosis of polymyositis is frequent in LGMD2B (1). The clinicians must maintain a high index of suspicion in front of a steroid resistant polymyositis (2): prolonged steroid treatment associated with immunosuppressive drugs should be avoided in refractory polymyositis if diagnosis is uncertain, because of their many associated adverse side effects. It is possible that steroids were not only unsuccessful in these two cases but might have worsened their outcome. Both cases had subacute onset in early teens. The second case, an active soccer player at age 14, presented a sudden onset of weakness with high CK, myalgias, swollen legs, and his first muscle biopsy was mistaken for a myositis, but clinical progression and muscle CT appearance were peculiar. Muscle biopsy was studied by Western blotting that gave in both cases the diagnosis of dysferlin deficiency. The disease progression is highly variable and additional factors as muscle fatigue, extent of autophagy and strenuous physical activity, that produce different types of physical stress, could determine different muscle injury and recovery. In agreement with previous study (3) an inflammatory response is seen in most dysferlinopathy cases. Rawat et al. (4) supposed that not only immune cells but also muscle cells can participate in inflammatory process. Attenuated and persistent muscle regeneration and release of chemotactic agent might also play a role (4). The amount of regenerating fibers in dysferlinopathy is high (5, 2) and may be similar to myositis. The exact nature of the trigger and the molecular pathways that initiate and perpetuate muscle fiber damage and dysfunction in LGMD2B is still unclear.

A reliable method for dysferlinopathy diagnosis is western blotting and subsequent genetic screening that yielded in our two cases both mutations in the dysferlin gene allowing a precise prognosis and genetic counselling. Although clinical diagnostic clues for dysferlinopathy diagnosis (6) have been developed the protein test remains a simple and inexpensive way to perform a rapid genetic diagnosis since all cases with absent protein were found subsequently to have pathogenic mutations (7).

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