Available online at www.sciencedirect.com

ScienceDirect

Neuromuscular Disorders 26 (2016) 662–665

www.elsevier.com/locate/nmd

Case report

Becker muscular dystrophy due to an intronic splicing mutation inducing a dual dystrophin transcript

Alice Todeschini [a], Francesca Gualandi [b], Cecilia Trabanelli [b], Annarita Armaroli [b], Anna Ravani [b], Marina Fanin [c], Silvia Rota [a], Luca Bello [c], Alessandra Ferlini [b,d], Elena Pegoraro [c], Alessandro Padovani [a], Massimiliano Filosto [a,*]

[a] *Center for Neuromuscular Diseases and Neuropathies, Unit of Neurology, ASST "Spedali Civili", University of Brescia, Brescia, Italy*
[b] *Department of Medical Sciences, Logistic Unit of Medical Genetics, University- Hospital of Ferrara, Italy*
[c] *Department of Neurosciences, University of Padua, Padua*
[d] *Dubowitz Neuromuscular Centre, Developmental Neuroscience Programme, UCL Institute of Child Health, London, UK*

Received 23 November 2015; received in revised form 24 July 2016; accepted 12 August 2016

Abstract

We describe a 29-year-old patient who complained of left thigh muscle weakness since he was 23 and of moderate proximal weakness of both lower limbs with difficulty in climbing stairs and running since he was 27. Mild weakness of iliopsoas and quadriceps muscles and muscle atrophy of both the distal forearm and thigh were observed upon clinical examination. He harboured a novel c.1150-3C>G substitution in the *DMD* gene, affecting the intron 10 acceptor splice site and causing exon 11 skipping and an out-of-frame transcript. However, protein of normal molecular weight but in reduced amounts was observed on Western Blot analysis. Reverse transcription analysis on muscle RNA showed production, via alternative splicing, of a transcript missing exon 11 as well as a low abundant full-length transcript which is enough to avoid the severe Duchenne phenotype. Our study showed that a reduced amount of full length dystrophin leads to a mild form of Becker muscular dystrophy. These results confirm earlier findings that low amounts of dystrophin can be associated with a milder phenotype, which is promising for therapies aiming at dystrophin restoration.

Keywords: Dystrophinopathy; Dystrophin; Duchenne muscular dystrophy; Becker muscular dystrophy; Splicing mutation; Dystrophin amount

1. Introduction

Dystrophinopathies are X-linked recessive disorders caused by mutations in the *DMD* gene. Duchenne muscular dystrophy (DMD) is the most severe phenotype of the disease, while Becker muscular dystrophy (BMD) is a later onset form, characterised by phenotypic variability ranging from severe muscle involvement to mild progressive muscular dystrophy with ambulation maintained into the teenage and adult years, or to milder forms including asymptomatic hyperCKemia (CK: Creatine phosphokinase) [1]. X-linked dilated cardiomyopathy (XLCM) is characterized by rapidly progressive heart pathology without muscular weakness [1].

The *DMD* gene maps on chromosome Xp21 and its product, dystrophin, is a membrane-associated protein present in muscle cells and other cells/tissues, composed of a N-terminal actin-binding domain, a large rod domain and a C-terminal domain that binds to dystroglycan and other proteins [1,2]. Dystrophin is part of a large protein complex that has mechanical, stabilising and signalling roles in mediating interactions between the cytoskeleton, membrane and extracellular matrix [1,2].

Deletions and duplications are the most common changes in the *DMD* gene, but point mutations (including nonsense, missense and splice mutations) have also been described [2,3]. Although exceptions do exist, the most useful rule for predicting whether a mutation will result in a severe or mild phenotype is the "reading frame rule" [3,4]. Frameshifting mutations (any types), which result in downstream multiple stop codons and produce no or small, unstable proteins, cause most of the DMD cases. In-frame mutations result in a

* Corresponding author. Unit of Neurology, ASST "Spedali Civili", University of Brescia, Pz.le Spedali Civili 1, Brescia 25100, Italy. Fax: 0039 030 3384086.
E-mail address: massimiliano.filosto@unibs.it (M. Filosto).

http://dx.doi.org/10.1016/j.nmd.2016.08.007
0960-8966/

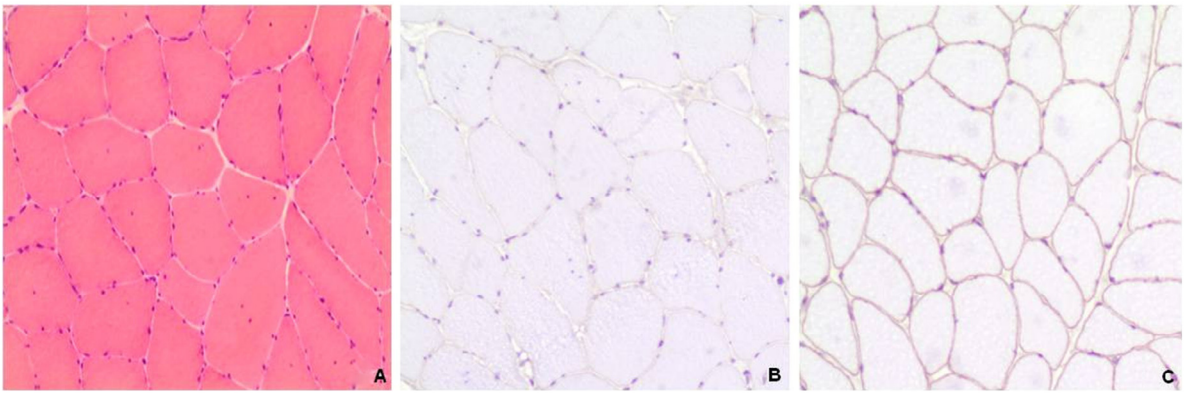

Fig. 1. 1A: Muscle fibre size variability, fibres with internal nuclei and mild focal endomysial fibrosis on Hematoxyline–Eosine staining (magnification 20×). 1B and 1C: Immunohistochemical study by using anti-dystrophin antibodies revealed a globally weak staining with antibodies against the C-terminal region of the protein (B). Immunoreactivity with antibodies against rod domain was normal (C).

quantitatively and qualitatively altered protein with partial wild-type function and usually in a BMD phenotype.

We here report a novel, intronic mutation causing exon 11 skipping in part of the dystrophin transcript. This skipping induces an out-of-frame transcript. However, a low amount of a wild type dystrophin is detectable in skeletal muscle, and the patient presents with a mild late-onset phenotype.

2. Case presentation

A 29-year-old male patient complained of left thigh muscle weakness since he was 23. Previously, he was a semi-professional football player. When he was 27, he started complaining of moderate proximal weakness in both lower limbs, with difficulty in climbing stairs and running.

At the neurological examination, the patient leaned on his thighs to get up from a squatting position and from sitting on the ground. Gower's sign was positive. He presented weakness of iliopsoas and quadriceps muscles (Medical Research Council scale for muscle strength: 4−/5) and muscle atrophy of both the distal forearm and thigh. No involvement of mimic muscles and extensors/flexors of the head were observed; tactile, pin-prick and vibratory sensation were normal; tendon reflexes were normal; no scapular winging, hypertrophy of calf muscles, myotonia, fasciculations, dysphagia, dysphonia, slurred speech, sphincter disturbances, hearing loss or reduction in visual acuity were present.

Serum CK levels were increased to 8–10 times the normal values (1600–1900 U/L; n.v. <190 U/L).

Electromyography recorded myopathic changes mostly in the proximal muscles of the limbs; electroneurography was normal. Standard and Holter electrocardiography were normal. Echocardiography showed slight concentric hypertrophy of the left ventricular walls and minimal left atrial enlargement with global preserved systolic function.

Neurological family history was unremarkable.

2.1. Muscle biopsy

Deltoid muscle biopsy showed a mild myopathy with few necrotic fibres, marked fibre size variability with atrophic and hypertrophic fibres, internal nuclei, rare fibre splitting and only mild focal endomysial fibrosis (Fig. 1A). Immunohistochemical analysis with anti-dystrophin antibodies (Novocastra Leica Biosystems) revealed a globally weak staining with antibodies against the C-terminal region of the protein (Fig. 1B). Immunoreactivity with antibodies against rod domain was present (Fig. 1C).

Immunohistochemical studies using anti alpha-sarcoglycan (Santacruz Biotechnology), gamma-sarcoglycan (Novocastra Leica Biosystems), beta-sarcoglycan (Novocastra Leica Biosystems), delta-sarcoglycan (Novocastra Leica Biosystems), caveolin-3 (Santacruz Biotechnology), dysferlin (Novocastra Leica Biosystems), alpha-dystroglycan (Santacruz Biotechnology), merosin (Novocastra Leica Biosystems), emerin (Novocastra Leica Biosystems) and lamin A/C (Novocastra Leica Biosystems) antibodies were normal (data not shown).

Western Blot (WB) analysis was conducted. Dystrophin amount was calculated by densitometric analysis of the corresponding band at approximately 420 kDa, normalised to myosin content as determined on post-transfer Coomassie-stained gel, and expressed as a percentage of the control.

WB showed a dystrophin protein of normal molecular weight but in reduced amounts (in the order of 7–30%) by using antibodies directed against the C-terminal (Fig. 2) and rod domain, and a secondary partial defect of alpha-sarcoglycan (data not shown).

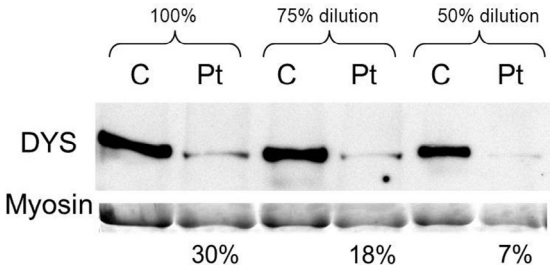

Fig. 2. WB with a dilution series showed reduced amount of a dystrophin protein of normal molecular weight (in the order of 7–30%) using antibodies directed against the C-terminal. DYS: dystrophin; C: control; Pt: patient.