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Case report

Anoctamin 5 muscular dystrophy associated with a silent p.Leu115Leu mutation resulting in exon skipping

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Abstract

We report a 45 year-old patient with an asymmetrical proximal muscle weakness affecting the quadriceps muscle of the right leg starting at the age of 32 years. CK was 25-fold increased. MRI of the legs showed signs of fatty degeneration more pronounced in the right side. Biopsy of a thigh muscle showed dystrophic pattern and amyloid deposition in blood vessel walls. The coding region and exon/intron boundaries of the *ANO5* gene were amplified and sequenced. The common c.191dupA mutation and a silent novel p.Leu115Leu (c.345G>A) variant were identified. This silent variant was listed neither in the LOVD database nor in the SNP database. To evaluate the pathogenicity of the novel silent mutation in *ANO5*, cDNA analysis was performed that demonstrated skipping of exon 6. So far, no case with a silent mutation leading to abnormal splicing has been identified in Anoctamin 5 muscular dystrophy. Present findings emphasize that cDNA analysis should be done if a silent variant is not annotated in the databases. In Anoctamin 5 muscular dystrophy a molecular diagnosis is even more important as protein investigation through Western blotting or immunohistochemistry is not yet established.

Keywords: Anoctamin 5; Dysferlin; Mutation; Silent mutation; Exon skipping

1. Introduction

Anoctamin 5 belongs to the Anoctamin protein family that is comprised of at least ten proteins. Each of these proteins consists of eight transmembrane domains and a DUF590 domain [1]. These proteins are reported to act as calcium-activated chloride channels. Exact function of anoctamin 5 is still unknown. However, in patients with non-dysferlin limb-girdle muscle dystrophy 2L (LGMD2L) and Miyoshi myopathy, sarcolemmal lesions and defective membrane repair has been identified suggesting that anoctamin 5 may function in muscle membrane repair [2,3].

In recent years, recessive mutations in the Anoctamin 5 gene (*ANO5*, OMIM 608662) have been reported in patients with LGMD2L and distal non-dysferlin *Miyoshi myopathy* [3–12]. The mutations reported in *ANO5* include missense-, nonsense-, insertion-, deletion- and splice-site- mutations. A c.191dupA mutation is found to be frequent in Northern European patients with LGMD2L, suggesting a founder effect in the Northern European population [5]. Dominant mutations in *ANO5* are associated with gnathodiaphyseal dysplasia (*GDD*, OMIM 166260) [13].

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So far, no silent point mutation that affects normal splicing has been reported in *ANO5*. However, there are several reports on exon skipping associated with silent point mutations in other disorders [14–17]. We report the first case of a silent mutation that leads to exon skipping in Anoctamin 5 muscular dystrophy.

2. Case report

A 45-year old German patient complained of muscle mass loss and muscle weakness mainly affecting the right leg. The patient was a football player in his school days and later actively played tennis. At the age of 32 years, during sports, he recognized mild leg muscle weakness. At the age of 37 years, he recognized difficulties to climb up stairs due to weakness in the right leg and he also noticed progressive atrophy of the right thigh. None of his 4 siblings (one brother died of an accident at age 33 years) had muscular symptoms. His parents and his two sons were also unaffected.

Clinical examination (Fig. 1A and B) showed marked atrophy in right thigh (mainly, distal portion of the vastus lateralis muscle) and a minor degree of atrophy in the medial head of the right gastrocnemic muscle. No muscle weakness was seen in the arms. In the legs, weakness was present in following muscles: hip adduction both sides (Medical Research Council [MRC] 4+), right hip extension (MRC 4+), hip flexion both sides (MRC 4), knee flexion both sides (MRC 4+), right knee extension (MRC 3). Serum CK level was increased up to 25-fold.

MRI of the upper legs revealed bilateral symmetrical fatty degeneration of the gluteus muscles and asymmetrical fatty degeneration of the adductor muscles, quadriceps muscle and biceps femoris muscle affecting mainly the right side (Fig. 1C). MRI of the lower legs showed bilateral fatty degeneration of the medial head of the gastrocnemic muscle on both sides (Fig. 1D). MRI of upper arms was normal. There was no cardiac and respiratory involvement.

Conventional myohistological examination of a formalin fixed biopsy of the left quadriceps muscle showed a myopathic pattern (Fig. 2A) with myophagic reactions but no endomysial mononuclear cells (Fig. 2B). Additionally, amyloid deposits within the blood vessel walls were seen by Congo red and Thioflavin S staining (Fig. 2C). There were no signs of inflammation.

Immunohistochemical staining with an antibody against dysferlin showed markedly reduced immunoreactivity at muscle fiber membranes but was diffusely increased in the cytoplasm of many fibers (Fig. 2D).

3. Materials and methods

Genomic DNA was extracted from peripheral blood of the patient and his parents using DNA extraction kit (Qiagen GmbH, Germany). Direct sequencing of the coding regions of the *ANO5* including exon–intron boundaries was performed after PCR amplification (primer sequences can be provided upon request).

Additionally, direct sequencing of the coding region of the dysferlin gene (*DYSF*) including exon–intron boundaries was also performed as described [18]. MLPA analysis was performed to identify duplications and deletions in the *DYSF*.

For cDNA analysis, total RNA was extracted from the blood of the patient using PAXgene RNA extraction kit (Qiagen GmbH, Germany). cDNA was obtained by RT-PCR (Qiagen GmbH, Germany) using following set of primers corresponding to *ANO5* that amplified a part of exon 5, whole exon 6 and a part of exon 7 resulting in an amplified product of 367 bp (forward primer:

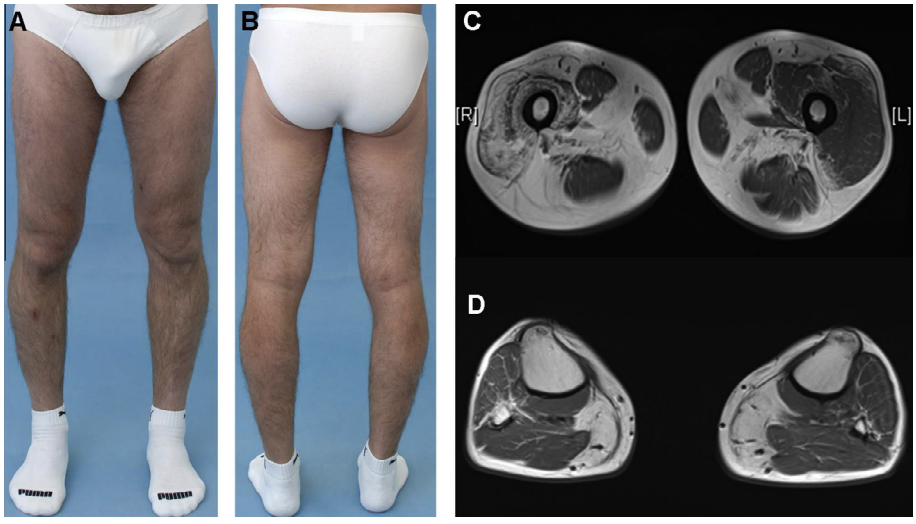

Fig. 1. Reported patient with anoctamin 5 muscular dystrophy with atrophy of the right vastus lateralis muscle and right calf (A) front view of legs, (B) rear view of legs, (C) MRI (T1 weighted) of thighs showing asymmetrical fatty degeneration on the adductor muscles, quadriceps muscle and biceps femoris muscle prominently on right side and (D) MRI (T1 weighted) of lower legs showing fatty degeneration in the medial head of the gastrocnemius muscle on both sides.