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Dysferlinopathy in the Jews of the Caucasus: A frequent mutation in the dysferlin gene

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Abstract

Dysferlin encoding gene (DYS) is mutated in the autosomal recessive disorders Miyoshi myopathy, Limb Girdle Muscular Dystrophy type 2B (LGMD2B) and distal anterior compartment myopathy, causing dysferlin deficiency in muscle biopsy. Three ethnic clusters have previously been described in Dysferlinopathy: the Libyan Jewish population originating in the area of Tripoli, Italian and Spanish populations. We report another cluster of this muscular dystrophy in Israel among Jews of the Caucasus region. A genomic analysis of the dysferlin coding sequence performed in patients from this ethnic group, who demonstrated an absence of dysferlin expression in muscle biopsy, revealed a homozygous frameshift mutation of G deletion at codon 927 (2779delG) predicting a truncated protein and a complete loss of functional protein. The possible existence of a founder effect is strengthened by our finding of a 4% carrier frequency in this community. These findings are important for genetic counseling and also enable a molecular diagnosis of LGMD2B in Jews of the Caucasus region.

Keywords: Dysferlin; LGMD2B; Muscular dystrophy; Founder effect

1. Introduction

Limb Girdle Muscular Dystrophy type 2B (LGMD2B), Miyoshi myopathy (MM) and distal anterior compartment myopathy are autosomal recessive muscular disorders caused by mutations in the dysferlin gene (DYSF) [1,2]. Dysferlin deficiency is demonstrated in muscle biopsy by immunohistochemistry and immunoblotting. The DYSF gene is located on chromosome 2p13, contains 55 coding exons and spans 150 kb of genomic DNA [3,4]. The transcript is 6.3 kb large and is mainly expressed in skeletal muscle and heart [5]. The protein is involved in calcium dependent membrane fusion step of the muscle fiber repair process [6,7]. No hot spot has been identified, and missense, nonsense and frameshift mutations have all been reported (Leiden Muscular Dystrophy database http://www.dmd.nl). About 400 allelic variants were described, 70% base substitutions and 20% small deletions consequently mutation analysis of DYSF remains a time-consuming task. Three ethnic clusters have been reported in LGMD2B: Libyan Jews [1,8], Italian [9] and Spanish [10] populations with a clear founder effect. In the Libyan Jews there is only a single DYSF mutation while in the Italy and Spain other mutations were found.

We have lately noticed another potential ethnic cluster of Dysferlinopathy in the Israel among Jews originating from the Caucasus Mountains region. Jews from the area

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bordered on the south by Anatolia and Iran in Asia, on the west by the Black Sea, on the east by the Caspian Sea and on the north by Russia belong to a highly inbred community, thus we assumed a founder effect and a common mutation in Dysferlinopathy patients originating from this geographical area.

The aim of this study was to perform a genomic analysis of the dysferlin coding sequence in this community.

2. Materials and methods

2.1. Patients

Ten patients from eight families originating from the Caucasus affected by muscular dystrophy were examined by neurologists from four Medical Centers in Israel. An informed consent was obtained from all patients, as well as from the healthy individuals. In three families, various degrees of consanguinity were established. At least one subject in every family had a muscle biopsy that confirmed the diagnosis of Dysferlinopathy. All patients had serum CK measurements and had had an EMG study at some stage of the disease.

Mean age at onset was 21.5 ± 6.5 years (range 10–30 years). All patients reported good physical condition before the onset of disease. They had gained normal milestones and were able to participate normally in physical activity at school. In all patients, weakness was noted initially in the legs. In four patients, initial symptoms were related to distal weakness. Two had inability to stand on tiptoes, one inability to stand on heels, and one had feet drop. In three patients, early symptoms were related to proximal leg weakness, mainly difficulty climbing stairs and rising from chair, with no clinical evidence of distal muscle weakness initially. Two patients had both proximal and distal involvement initially. One patient had no complaints at onset, but hyper CKemia was discovered by incident. On examination he was unable to stand on tiptoes only.

Upper limb involvement was noted in six patients. It occurred 5–11 years after the onset, at a mean age of 25 years.

On examination the weaker proximal muscles were the iliopsoas, quadriceps and hamstrings. At advanced stages the feet and toes dorsiflexors were usually weaker than the gastrocnemius. In the upper limbs the biceps was usually initially affected, but in a short time there was no difference among the proximal muscles of the upper limbs (deltoid, biceps, and triceps). The shoulder girdle musculature was less involved and scapular winging was not observed. The distal muscles of the upper limbs were usually spared.

In all patients, serum CK levels were 15–30 times the upper limit of normal on repeated tests throughout. EMG in all tested patients showed no evidence of spontaneous activity, even in the distal muscles involved at an early stage. Motor units were usually of small amplitude and short duration, with early recruitment, suggesting a myopathic pattern.

2.2. Protein analysis

Western blot analysis was performed according to published procedures [5,6] using 4.5% acrylamide gel and a 4 h electrophoretic run. The primary antibodies used were NCL-Hamlet (Novocastra, Newcastle upon Tyne, UK), the secondary antibodies were goat anti-mouse from Jackson.

2.3. Genotyping

Genomic DNA was extracted from peripheral blood by the Puregene kit (Gentra, Minneapolis, USA), according to the manufacturer's instructions. Genomic DNA was used as a template for PCR amplification of each of the 55 exons of the DYSF gene. The primer sets and the amplification conditions are described in Table 1. The reaction was performed in a 50 μL volume containing 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$ 250 μM dNTPs, 1 μM of each primer, 100 ng of genomic DNA and 1.25 U of AmpliTaq Gold DNA polymerase (Perkin-Elmer Applied Biosystem) with an initial denaturation step of 10 min at 95 °C to activate the polymerase followed by 35 cycles of 94 °C; 15 s, 60 °C; 45 s, 72 °C; 45 s and a final elongation of 10 min at 72 °C. Predicted amplicon sizes were confirmed by agarose gel electrophoresis. The amplified PCR products were purified with the ExoSapIT kit (Amersham Pharmacia Biotech, Amersham, Buckinghamshire, UK), according to the manufacturer's instructions, and sequenced with fluorescently labeled dedeoxynucleotide terminators and an Applied Biosystem 373A automated sequencer. Sequences were compared with the DYSF transcript (ENST00000258104).

2.4. Carrier detection

A mismatch PCR was performed using the following primer set: forward: 5′-GCC CCT CGG CCG GCT GGA CCC GG-3′ reverse: 5′-CAG TCC TGG GAG AGT TCA GC-3′. PCR conditions were the same as mentioned above, including 0.01% gelatin, 3.5% formamide, 10% glycerol and 75 μM 7-deaza guanine. PCR product (206 bp) was digested with SmaI, according to the manufacturer's instructions. Normal PCR products were digested into $185 + 21$ while carriers demonstrated a pattern of $206 + 185 + 21$. Mutants were not digested.

3. Results

3.1. Western blot analysis

Western blot analysis revealed a complete absence of the dysferlin signal while merosin (as a reference