Clinical Casebook

Milder course in Duchenne patients with nonsense mutations and no muscle dystrophin


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Abstract

Duchenne muscular dystrophy (DMD), a severe and lethal condition, is caused by the absence of muscle dystrophin. Therapeutic trials aiming at the amelioration of muscle function have been targeting the production of muscle dystrophin in affected Duchenne patients. However, how much dystrophin is required to rescue the DMD phenotype remains an open question. We have previously identified two exceptional golden retriever muscular dystrophy (GRMD) dogs with a milder course despite the total absence of muscle dystrophin. Here we report two unusual patients carrying nonsense mutations in the DMD gene and dystrophin deficiency but with an unexpectedly mild phenotype. Three reported polymorphisms, respectively in genes LTBP4, SPP1 and ACTN3 were excluded as possible DMD genetic modifiers in our patients. Finding the mechanisms that protect some rare patients and dogs from the deleterious effect of absent muscle dystrophin is of utmost importance and may lead to new avenues for treatment. Importantly, these observations indicate that it is possible to have a functional large muscle even without dystrophin.

Keywords: Duchenne dystrophy; Milder progression; Nonsense mutation; No dystrophin

1. Introduction

Duchenne muscular dystrophy (DMD) is a lethal X-linked condition caused by mutations in the dystrophin gene which results in the absence of muscle dystrophin protein. The course is usually severe and very similar in affected patients. Onset occurs around 2 to 3 years of age and without careful management, loss of ambulation between 9 and 12. On the other hand, in Becker muscular dystrophy (BMD), there is a wide variability (intra and inter familial) in the severity of the phenotype which has been mainly associated with the site of the deletion and to the amount of muscle dystrophin. Therefore, the quantity/quality of muscle dystrophin has been strongly associated with appropriate muscle function.

Some exceptional patients [1] and dystrophic animal models, however, deserve special attention. For instance, understanding why the mdx mice, the murine model for DMD is almost asymptomatic, despite total absence of dystrophin has been a great challenge. Possible explanations are their smaller size and shorter lifespan. However, we have identified two exceptional golden retriever muscular dystrophy (GRMD) dogs with no muscle dystrophin and a very mild phenotype [2]. Here we report two very rare examples of patients carrying nonsense mutations and a milder phenotype despite the absence of muscle dystrophin. These observations indicate that it is possible to have a functional large muscle even without dystrophin.
2. Clinical and laboratory exams

2.1. Case 1

Two half-brothers called our attention due to their strikingly discordant phenotype (Fig. 1A). Onset of symptoms in the younger brother (II) was at age 3, a DMD diagnosis was established at age 7, and he was wheelchair-bound at age 9. His serum CK is 3260 U/l (normal up to 189 U/l). His older half-brother (I) was noticed to have some weakness at age 13, when his younger brother was diagnosed. Currently, at age 15, he

Fig. 1. (A) The two brothers, illustrating their calf hypertrophy. (B) The 15 year-old isolated DMD patient; C-Muscle histological and immunohistochemical analysis for dystrophin, using DYS 2. (C) Terminal antibody. (D) Western blot analysis showing the reaction for dystrophin N-terminal antibody (DYS3, Vector), rod domain antibody (DYS1, Vector) and C-terminal antibody (DYS2, vector). A reaction with antibody for alpha actinin 3 (ACTN3, generous gift from Dr. Alan Beggs, Boston, USA), showed the presence of the protein in the 3 analyzed patients. (C) normal control muscle, Myosin-band observed in the Ponceau S pre-stained blot.
is only mildly affected, with discrete calves hypertrophy, some difficulties for running and climbing stairs but with normal ability for walking. His serum CK is 3620 U/l.

MLPA (multiplex ligation dependent probe amplification) DNA analysis revealed that both brothers carry an “out of frame” duplication in exon 2 of the dystrophin gene which was not present in the mother blood lymphocytes, indicating a gonadal mosaicism.

Despite the clinical differences, muscle histology analyzed in blind test showed a similar pattern in both brothers with variation in fiber size, degeneration, splitting fibers, centrally located nuclei and connective tissue replacement (Fig. 1C).

Dystrophin immunostaining with at least 3 antibodies against the N-terminal, rod domain and C-terminal, showed a negative pattern, with a cluster of 6–8 positive fibers (reverting fibers) per section, in both of them (Fig. 1C). Merosin was positive, and sarcoglycans were faints, as observed in most DMD dystrophin deficient patients.

When normalized for the myosin content and compared to normal controls in the same blot, dystrophin amount through WB revealed a very faint band, of less than 5% of normal and very similar in both of them, using an antibody against the rod domain. No dystrophin bands were identified with the N-terminal and C-terminal antibodies (Fig. 1D).

Therefore, although the two brothers exhibited equally low amounts of dystrophin they presented different phenotypes.

2.2. Case 2

This isolated DMD patient who, at age 16, is able to walk without difficulties and climb stairs with the aid of the banister was first seen in our center at age 7. At that time, he had a typical DMD phenotype with difficulties for climbing stairs, running or raising up from the floor (Fig. 1B). His serum CK was grossly elevated (30,756 U/l). DNA analysis through MLPA revealed the presence of an out-of-frame deletion, encompassing exons 51–54, in the dystrophin gene. Currently, although he has a visible muscle dystrophin band through western blot, it is important to point out that all these exceptional BMD patients with apparently nonsense mutations had some amount of dystrophin in muscle biopsy in accordance with their milder phenotype. More recently, a DMD patient with significant growth delay due to corticotherapy, who was also still ambulant at age 19, was reported [5]. However, the two 15 and 16 year old patients with a milder course here reported have normal growth.

A family with clinically discordant relatives have been previously reported by us in a maternal uncle and his nephew. But, differently from the present cases proteins and molecular analyses revealed that the uncle with a milder course had some muscle dystrophin as a result of an alternative splicing in the dystrophin gene [6,7]. Kesari et al. [8] reported a high rate of exceptions in the reading-frame rule in a study of 75 patients, mainly in BMD patients involving the N-terminal region of the gene. This is explained by the high incidence of 5’ gene deletions/duplications in a region known as a hotspot for exceptions due to complex splicing patterns [8]. However, it is important to point out that all these exceptional BMD patients with apparently nonsense mutations had some amount of dystrophin in muscle biopsy in accordance with their milder phenotype. More recently, one more patient with a nonsense mutation in exon 2 and a milder course was reported [9] but he presented a visible muscle dystrophin band through western blot, differently from our two here reported cases.

In the two milder affected patients, DNA mutations were compatible with dystrophin deficiency observed in their muscles, which showed a very similar pattern in the three of them, as well as in more than 300 DMD patients with nonsense mutations we have analyzed in our center so far. Therefore, the milder phenotype could not be related to differences in the amount of expressed protein. Consistently with the muscle dystrophin deficiency, all complementary exams, in particular muscle histopathology, and other studied proteins showed a very similar pattern in the two half-brothers as well as in the other unrelated 16-years old patient, despite their clinical difference. In short, in both families there is a

Analysis of the LTBP4 haplotype in the three patients showed the following genotypes: IV-AT-AT-MT, for case I mildly affected brother, II-AT-AT-TT for case II more severely affected brother, and IV-AT-AT-MT for case III. Therefore, all 3 cases were heterozygous for this haplotypes.

Analysis of the SNP-66T>G (rs28357094) in the osteopontin gene (SPP1), where the G allele (GG or TG) has been apparently associated with a more severe phenotype, showed the TG, TT and TT genotypes in patients I, II and III, respectively.

3. Discussion

Patients with a discordant Duchenne and Becker phenotype belonging to the same family have been previously reported by us and others. In one situation, the milder progression was associated with growth hormone deficiency [3,4]. More recently, a DMD patient with significant growth delay due to corticotherapy, who was also still ambulant at age 19, was reported [5]. However, the two 15 and 16 year old patients with a milder course here reported have normal growth.

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