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Case report

Becker muscular dystrophy patients with deletions around exon 51; a promising outlook for exon skipping therapy in Duchenne patients

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ABSTRACT

Theoretically, 13% of patients with Duchenne muscular dystrophy may benefit from antisense-mediated skipping of exon 51 to restore the reading frame, which results in the production of a shortened dystrophin protein. We give a detailed description with longitudinal follow up of three patients with Becker muscular dystrophy with in-frame deletions in the *DMD* gene encompassing exon 51. Their internally deleted, but essentially functional, dystrophins are identical to those that are expected as end products in DMD patients treated with the exon 51 skipping therapy. The mild phenotype encourages further development of exon 51 skipping therapy.

1. Introduction

Mutations in the dystrophin-encoding *DMD* gene on the X chromosome result generally in Duchenne muscular dystrophy (DMD) if the mutation is out-of-frame and in Becker muscular dystrophy (BMD) if the mutation preserves the translational reading frame. In BMD patients an altered form of dystrophin is present, whereas in DMD patients dystrophin is virtually absent in muscle fibers. Absence of dystrophin in a muscle biopsy has an unfavorable prognosis, as DMD patients become wheelchair bound before the age of 13. The clinical phenotype in BMD is milder with a large variation in clinical severity.

Currently, new therapeutic strategies, such as antisense-mediated exon skipping, are in an early phase of clinical trials and have the potential to change the course of the DMD disease dramatically. In our recent study, intramuscular injection of an antisense oligonucleotide (AON) induced skipping of exon 51 and restored the disrupted open reading frame and therefore the production of dystrophin in four DMD patients with deletions of exons 48–50, 49–50, 50 and 52, respectively [1]. Clinical trials with systemic administration of AON are taking place. If successful, therapeutic skipping using an AON that targets exon 51 can stop further muscle wasting, resulting in a clinical phenotype like BMD. This AON can be applied in about 13% of the DMD patients [2]. Therefore, focusing on the functionality of the probable end product through studying corresponding Becker phenotypes is useful and will provide information for patients eligible for this new therapy. This would concern the in-frame deletions of exon 45–51, 47–51, 48–51, 49–51, 50–51, 51–52, 51–58, 51–61 and 51–63 that all are predicted to result in a BMD phenotype.

We describe the clinical phenotype in two BMD pedigrees in the Netherlands carrying deletions including exon 51. We also provide a review of BMD patients with these deletions reported in the literature.

2. Patients and methods

2.1. Methods

Since the availability of DNA diagnostics in 1984, more than 1500 Dutch DMD/BMD families have been tested in our laboratory. The laboratory database was searched to find patients with in-frame deletions including exon 51. After obtaining informed consent, data on clinical history and neurological examinations were extracted from clinical files and/or obtained directly from the patients by the authors (C.S. and A.H.-E.).

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Data from additional patients were collected by searching the literature and by consulting the international DMD database at the Leiden Muscular Dystrophy pages (http://www.DMD.nl) [3].

Where ever possible, additional information was obtained from the authors who have published since 1993 on patients with either a deletion of exon 45–51 or of exon 50–51 and submitted to the database.

3. Results

3.1. Description of Dutch pedigrees

In the Dutch population we found one pedigree with a deletion of exons 45–51 and one with a deletion of exons 50–51. Up until February 2009 no Dutch BMD patients had been registered with in-frame deletion of the exons 47–51, 48–51, 49–51, 51–52, 51–58, 51–61 and 51–63.

3.1.1. Family 1: deletion exons 45–51

Patient A1, born in 1962, had suffered from painful muscle cramps in his legs since childhood. Cramps were mostly provoked by exercise such as hiking or occured after he had bumped his leg. Cramps resulted in painful nodules and could last for one and a half hour. Sometimes his father had to carry him home from school. He did not participate in sports and did not like running. Neurological examination at the age of 14 showed no muscle weakness. A biopsy of the quadriceps muscle at this time showed dystrophic features, with groups of necrotic fibers and groups of regenerating fibers as well as local increase of endomysial fibrous connective tissue. In 1990 Western blotting showed a slightly reduced amount of dystrophin with a smaller molecular weight. DNA analysis identified an in-frame deletion of exons 45–51 in the *DMD* gene and confirmed the diagnosis of BMD. He now works as a truck driver and rarely suffers from cramps and is not limited in his daily activities, although he avoids jumping from his truck or climbing more than two stairs.

In childhood he had trouble concentrating, was hyperactive and attended a primary school for children with educational problems. Subsequently he succeeded in obtaining a certificate from a regular technical school.

A neurological examination in 2008 was unremarkable except for calf hypertrophy. His creatine kinase (CK) which had increased 50-fold in 1976 was only marginally increased in 2008 (243 with a reference value up to 200 U/l). Cardiac examination including echocardiography showed no abnormalities. MRI showed normal aspect of the shoulder muscles and the muscles of the leg. There were minimal fatty changes in the hip extensors (Fig. 1).

His maternal grandfather (patient A2), born 1913 and deceased 1993, carried the same mutation. He too had suffered from cramps in childhood and adolescence. At the age of 78 he was examined by the late Prof. HFM Busch, neurologist. The grandfather showed no neurological signs or symptoms of BMD and his CK values were normal.

3.1.2. Family 2: deletion exons 50–51

Patient B1, born in 1994, visited a pediatrician at the age of 8 because of hyperactive behavior. His past history was unremarkable including normal motor milestones; he was able to walk unsupported at the age of 18 months. Routine laboratory examination revealed increased transaminases and subsequently 8-fold increase in CK. Neurological examination in 2003 was unremarkable. DNA analysis showed an in-frame deletion of exons 50–51 in the *DMD* gene.

In 2008 he sporadically suffered from muscle cramps but did not complain about muscle weakness. He cycled to school every day for more than an hour each way, including riding uphill. He enjoyed climbing trees to help with pruning the branches, and played in a soccer team. Neurological examination showed normal muscle strength. There was no calf hypertrophy. ECG and echocardiography were normal. His mean time on a timed run test of 10 m was 2.6 s, (reference: mean time to run 9 m for healthy boys of 11 years is 2.5 ± 0.28 s) [4].

Behavior problems were diagnosed as attention deficit hyperactivity disorder (ADHD) by a child psychiatrist. Cognitive tests showed a subnormal IQ of 80 with performance IQ lower (77) than verbal IQ (90). For hyperactivity he was treated with methylphenidate (Ritalin) 54 mg/day, 5 days a week. He attended a primary school for special education. He now follows regular secondary education. He is still hyperactive during the weekend when he is off methylphenidate.

His mother carries the same BMD mutation. He has a healthy brother, born 1992, who has not been tested.

3.2. Patients from the DMD database at the Leiden Muscular Dystrophy pages and from published reports

The DMD gene variant database at the "Leiden Muscular Dystrophy pages, http://www.DMD.nl" [3] is a public repository of variants reported in literature or submitted directly to the database. We used

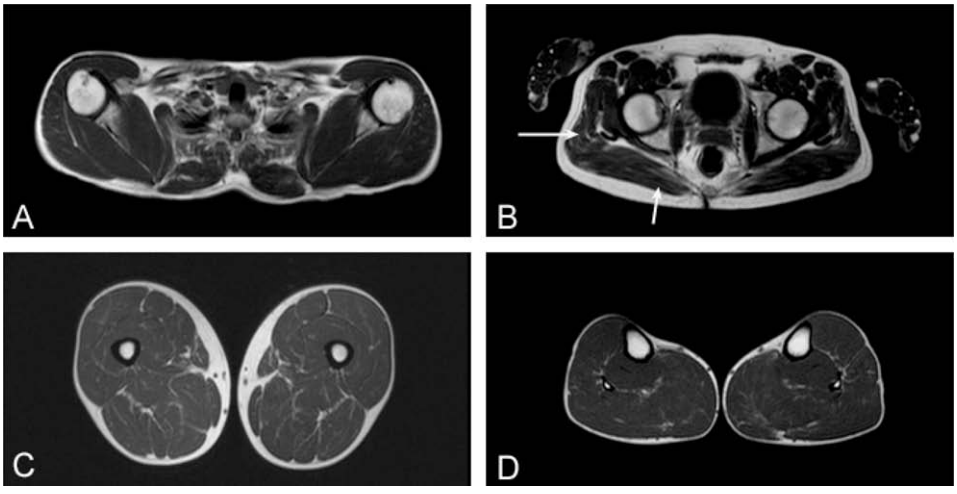

Fig. 1. MRI images of the muscles in patient A1. MRI axial T1-weighted images of the muscles in patient A1 at age 46 shows at the level of (A) the shoulder: normal volume and signal intensity of the muscles, (B) the hip joint: minimal fatty changes in the gluteal muscles (arrows), (C) upper leg: normal volume and signal intensity of the muscles and (D) lower leg: normal volume and signal intensity of the muscles.