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Chapter 5

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

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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.).
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]. Wherever 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 unsupervised 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.](http://www.DMD.nl)
this valuable resource to trace 57 patients with a deletion of exons 45–51 or 50–51, including our three patients. On February 3rd 2009 the DMD database listed 27 entries with a deletion of exons 45–51 (including 50 patients) and six entries with a deletion of exons 50–51 (7 patients); 21 of the 33 submissions were published reports. After contacting the authors, six patients were excluded for further analysis; in two patients further testing with MLPA (Multiplex Ligation-dependent Probe Amplification) refined the mutations originally detected by PCR to deletions of exons 45–54 and 49–51. One submitter reported that two patients could not be traced back in his database. Furthermore, two patients were excluded because they appeared to have a Duchenne phenotype; their genotype was tested with multiplex-PCR and might be not reliable enough. One submission appeared to be a monozygotic twin. All new findings were reported to the DMD database and used to update the records. In summary, 50 patients were traced using the information in the DMD gene variant database.

Our literature search identified six more patients with deletions of exons 45–51 [5–7] and six more patients with deletion exons 50–51 in the literature [8,9].

We made an overview of the 19 patients of whom we obtained clinical information (Tables 1 and 2). These 19 BMD patients came from 12 families. For 43 patients no further information was available.

### 4. Discussion

Our study shows that BMD patients with 45–51 or 50–51 in-frame mutations have a mild phenotype.

The clinical phenotype of the three Dutch patients, including the grandfather shows certain similarities. The course of the disease is mild with muscle cramps without muscle weakness and hyper-CK-emia being the main features. As adults these patients showed no clear evidence of progression of the disease with age. None of the patients experienced major limitations in daily life. Intolerance to exercise appears to restrict patient A1. Although both patients A1 and B1 have had behavioral or attention problems at primary school, they attended regular secondary school and patient A1 functions normally as an adult. Behavioral and attention

### Table 1
BMD patients with a deletion of exons 45–51.

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Reason referral</th>
<th>Age at presentation (yr)</th>
<th>Last examination at (yr)</th>
<th>Symptoms</th>
<th>CK (IU/L) highest</th>
<th>Cardiac echography</th>
<th>Test method</th>
<th>Result muscle biopsy</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>Muscle cramps</td>
<td>4</td>
<td>45</td>
<td>Cramps</td>
<td>2200</td>
<td>Normal</td>
<td>MLPA</td>
<td>Dystrophin present</td>
<td>Patient A1 present study</td>
</tr>
<tr>
<td>A2</td>
<td>Grandfather patient A1</td>
<td>78</td>
<td>78</td>
<td>Cramps</td>
<td>Normal</td>
<td>M-PCR</td>
<td>Dystrophin present</td>
<td>Patient A2 present study</td>
<td></td>
</tr>
<tr>
<td>A3</td>
<td>Cousin of patient A4</td>
<td>5</td>
<td>5</td>
<td>Contractures, mild proximal muscle weakness, mild calf hypertrophy</td>
<td>1700</td>
<td>M-PCR</td>
<td>Dystrophin present</td>
<td>Patient A2 present study</td>
<td></td>
</tr>
<tr>
<td>A4</td>
<td>Cousin of patient A4</td>
<td>8</td>
<td>8</td>
<td>No</td>
<td>2950</td>
<td>Normal</td>
<td>M-PCR</td>
<td>Dystrophin present</td>
<td>[7]</td>
</tr>
<tr>
<td>A5</td>
<td>Pain on exercise</td>
<td>7</td>
<td>7</td>
<td>No</td>
<td>896</td>
<td>Normal</td>
<td>M-PCR</td>
<td>Dystrophin present</td>
<td>[6]</td>
</tr>
<tr>
<td>A7</td>
<td>Pain on exercise</td>
<td>8</td>
<td>8</td>
<td>Pain, mild calf hypertrophy</td>
<td>1000</td>
<td>Normal</td>
<td>M-PCR</td>
<td>Dystrophin present</td>
<td>[21]</td>
</tr>
<tr>
<td>A9</td>
<td>Myoglobinuria</td>
<td>14</td>
<td>14</td>
<td>No</td>
<td>M-PCR</td>
<td>No muscle biopsy</td>
<td>Dystrophin present</td>
<td>[5] patient B-25</td>
<td></td>
</tr>
<tr>
<td>A10</td>
<td></td>
<td>10</td>
<td>10</td>
<td>No</td>
<td>2648</td>
<td>M-PCR</td>
<td>DMD database</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A11</td>
<td></td>
<td>4</td>
<td>7</td>
<td>Mild calf hypertrophy</td>
<td>3375</td>
<td>Normal</td>
<td>MLPA</td>
<td>No muscle biopsy</td>
<td>Kekou Kanavakis</td>
</tr>
<tr>
<td>A12</td>
<td></td>
<td>4</td>
<td>16</td>
<td>No</td>
<td>810</td>
<td>Normal</td>
<td>M-PCR</td>
<td>Dystrophin present</td>
<td>[22]</td>
</tr>
</tbody>
</table>

**Abbreviations used:** MLPA, Multiplex Ligation-dependent Probe Amplification; M-PCR, Multiplex Polymerase Chain Reaction; CK, Creatine Kinase.

### Table 2
BMD patients with a deletion of exons 50–51.

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Reason referral</th>
<th>Age at presentation (yr)</th>
<th>Last examination at (yr)</th>
<th>Symptoms</th>
<th>CK (IU/L) highest</th>
<th>Cardiac echography</th>
<th>Test method</th>
<th>Result muscle biopsy</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>Limping falling</td>
<td>8</td>
<td>13</td>
<td>Cramps</td>
<td>1533</td>
<td>Normal</td>
<td>MLPA</td>
<td>No muscle biopsy</td>
<td>Patient B1 present study</td>
</tr>
<tr>
<td>B2</td>
<td></td>
<td>2</td>
<td>4</td>
<td>Yes</td>
<td>1300</td>
<td>Normal</td>
<td>M-PCR</td>
<td>Dystrophin present</td>
<td>[8]</td>
</tr>
<tr>
<td>B3</td>
<td>Grandfather patient B2</td>
<td>69</td>
<td>69</td>
<td>No</td>
<td>Normal</td>
<td>M-PCR</td>
<td>M-PCR</td>
<td>[8]</td>
<td></td>
</tr>
<tr>
<td>B4</td>
<td>Great uncle of patient B2</td>
<td>55</td>
<td>55</td>
<td>No</td>
<td>M-PCR</td>
<td>M-PCR</td>
<td>M-PCR</td>
<td>[8]</td>
<td></td>
</tr>
<tr>
<td>B5</td>
<td>Cousin of mother of patient B2</td>
<td>28</td>
<td>28</td>
<td>No</td>
<td>M-PCR</td>
<td>M-PCR</td>
<td>M-PCR</td>
<td>[8]</td>
<td></td>
</tr>
<tr>
<td>B7</td>
<td>Maternal cousin DMD patient with another mutation</td>
<td>18</td>
<td>18</td>
<td>No</td>
<td>327</td>
<td>M-PCR</td>
<td>Dystrophin present</td>
<td>[9]</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations used:** MLPA, Multiplex Ligation-dependent Probe Amplification; M-PCR, Multiplex Polymerase Chain Reaction; CK, Creatine Kinase.
problems appear to occur more frequently in boys with Becker muscular dystrophy than in the general population [10].

Also the patients reported in the literature had a mild course of the disease. At older age they did not show any symptoms of BMD and had a normal result of cardiac examination [6,8]. The low prevalence of known in-frame mutations encompassing exon 51 in the Dutch population could well be an underestimation. Patients with a comparable mild phenotype as the ones we have described might not have been recognized as a possible BMD and may never have visited a neurologist or a pediatrician.

Our study clearly shows that previous publications are not always correct regarding the extent of the deletions reported. Until recently deletions were mainly detected using methods like Southern blotting, multiplex-PCR (Chamberlain and Beggs sets [11,12] and quantitative multiplex-PCR. MLPA [13] is much more accurate and ideally should be used to confirm the deletions and, more importantly regarding clinical outcome, to reliably determine its boundaries. Furthermore, only analysis at RNA level will be able to confirm that the translational reading frame remains intact. It should be noted that the public availability of the DMD database was an enormous help for our study. It gave a clear overview of the findings thus far and an easy resource to contact the groups having patients of interest. Of course it is unfortunate that not all data reported are correct, but the database itself is not to blame for this. To support the database we immediately reported additions and inconsistencies for inclusion or correction.

Gathering information about the phenotype of BMD patients has become relevant now that exon skipping therapies are being developed for DMD patients. Since exon skipping is mutation specific, each specific exon skip will result in a different shortened variant of dystrophin. Therefore, information on the best target exon for individual DMD patients may be found by examining BMD patients with different dystrophin variants. Since the first clinical trials are aimed at skipping exon 51, we have focused on BMD patients with a mutation encompassing this region. In fact, one of the DMD patients that was treated in the first human exon skipping trial [1] had a deletion of exon 50. After local treatment with antisense PRO051 the reading frame was restored and he produced dystrophin which should be similar to the dystrophin of patient B1 in our study. This demonstrates how one exon made the difference of being wheelchair bound at age 9 or climbing trees at age 14. The ultimate goal is to develop specific treatments to restore dystrophin production in DMD patients.

Besides, it is important to realize that these findings are mutations specific as a smaller in-frame deletion in this same area, such as a deletion of exons 48–49, results in X-linked dilated cardiomyopathy [14]. Also the in-frame deletions of exon 51–61 and 51–63 will most probably result in a moderate to severe phenotype, because they are close to the cysteine rich region (exon 64–70) which might affect the binding of dystrophin to β1-dystroglycan [15]. Mice with a deletion of such a cysteine rich region have muscles with severe dystrophic pathology [16]. Furthermore, a deletion of exons 45–55 is associated with a BMD phenotype with a prognosis of a favorable outcome [17], but can also be associated with X-linked dilated cardiomyopathy depending on the exact location of the breakpoints of the deletion [18,19].

Given the many different mutations, patients will benefit highly of multidisciplinary cooperation that results in databases with information on genotype as well as on phenotype [20]. For the dystrophinopathies good databases will facilitate and accelerate the inclusion of patients in clinical trials. This underlines the importance of setting up national patient registries, which can contribute to an international database. A good example of such a database is the TREAT NMD network of excellence (http://www.treat-nmd.eu/). In conclusion, the phenotype of BMD patients with a deletion of exons 45–51 and 50–51 appears to be mild. This is encouraging for the outcome of the exon 51 skipping trials and offers hope to DMD patients who are eligible for this therapy.

Acknowledgments

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References