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Clinical characterization of Becker muscular dystrophy patients predicts favourable outcome in exon-skipping therapy

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Abstract

Objective

Duchenne and Becker muscular dystrophy (DMD/BMD) are both caused by mutations in the *DMD* gene. Out-of-frame mutations in DMD lead to absence of the dystrophin protein, while in-frame BMD mutations cause production of internally-deleted dystrophin. Clinically, DMD patients loose ambulance around the age of twelve, need ventilatory support in their late teens and die in their third or fourth decade due to pulmonary or cardiac failure. BMD has a more variable disease course. The disease course of BMD patients with specific mutations could be very informative to predict the outcome of the exon skipping therapy, aiming to restore the reading-frame in DMD patients.

Methods

BMD patients with a mutation equaling a DMD mutation after successful exon skipping were selected from the Dutch Dystrophinopathy Database. Information about disease course was gathered through a standardized questionnaire. Cardiac data were collected from medical correspondence and a previous study on cardiac function in BMD.

Results

Forty-eight patients were included, representing 11 different mutations. Median age of patients was 43 (range 6-67). Nine patients were wheelchair dependent (at age 26 to 56 years). Dilated cardiomyopathy was present in 7/36 patients. Only one patient used ventilatory support. Three patients had died at the age of 45, 50 and 76 respectively.

Conclusions

This study provides mutation specific data on the course of disease in BMD patients. It shows that the disease course of BMD patients, with a mutation equaling a "skipped" DMD mutation, is relatively mild. This finding strongly supports the potential benefit of exon skipping in DMD patients.

Introduction

Duchenne and Becker Muscular Dystrophy (DMD/BMD) are X-linked muscular dystrophies caused by mutations in the *DMD* gene.[173] This gene encodes several isoforms of dystrophin, which are expressed primarily in muscle, brain and retina.[174] In DMD the absence of dystrophin in muscle leads to a quite uniform progressive disease course with patients loosing ambulation before the age of twelve, needing mechanical ventilation around the age of eighteen and leading to death in the third or fourth decade due to respiratory or cardiac failure, as the heart muscle is also involved.[7,8,14] In BMD partially functional muscle dystrophin is present, leading to a highly variable disease course, ranging from a phenotype only slightly less severe than in DMD patients to patients who remain ambulant throughout life.[19,21] This variation is at least partly thought to be explained by the location of the mutation in the *DMD* gene.[22,25,64] Currently, no long-term effective treatment for DMD and BMD exists. Several therapeutic interventions are being developed, of which exon skipping using antisense nucleotides is regarded the most promising.[170,175] Exon skipping restores the reading frame at the pre-mRNA level at the cost of an increased deletion size, resulting in the production of partially functional BMD-like dystrophin instead of a non-functional DMD-like dystrophin (Figure 1).[176] Thus, this therapy aims to alter a DMD phenotype into a BMD phenotype. BMD patients with a mutation equaling a DMD mutation after successful restoration of the reading frame represent a model of the potential beneficial effect of exon skipping in patients with DMD. We collected the clinical characteristics of BMD patients with in-frame deletions corresponding to the predicted result after successful exon skipping of out-of-frame DMD mutations. We postulate that the clinical course of BMD patients with in-frame deletions in our study reflects the result of a successful exon skipping treatment of DMD patients with the corresponding out-of-frame deletions.

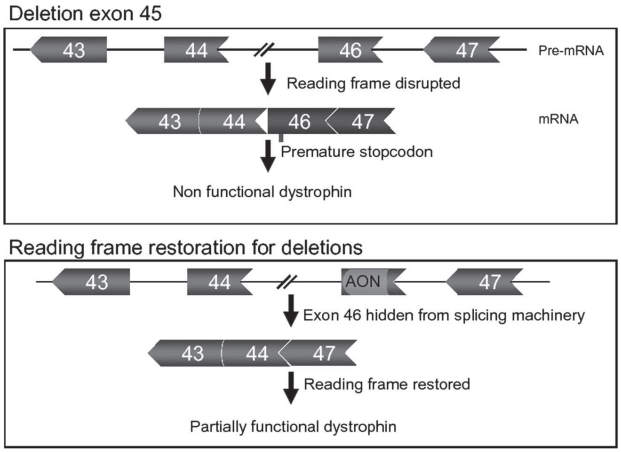

Figure 1. Exon-skipping technology.
A deletion of exon 45 results in a disruption of the reading frame of the dystrophin mRNA, causing a DNA phenotype. By 'hiding' exon 46 from the splicing machinery, this exon is excluded from the mRNA, resulting in a restoration of the reading frame. This leads to the production of partially functional dystrophin and a BMD phenotype.
AON: antisense oligonucleotide.

Patients and methods

Patients

Patients were selected from the Dutch Dystrophinopathy Database (DDD), which represents over 50% of the Dutch DMD and BMD population, and contains information on all TREAT-NMD mandatory and recommended items. Patients were recruited for this registry through Dutch patient organizations, physicians and internet (www.lumc.nl/duchenne). Information was gathered about medical history, disease course, education and family through a written standardized questionnaire. All patients provided written informed consent. The educational data were compared to that of Duchenne patients registered in the DDD as well as to the general Dutch population using information from 'Statistics Netherlands' (statline.cbs.nl). Patients were included in the present study when their mutation equaled the result of an out-of-frame DMD mutation that could be changed into an in-frame BMD mutation by skipping one exon. For clinical comparison, data were collected from the DDD of DMD patients with corresponding out-of-frame mutations.

The study was approved by the local ethical committee as part of the study "Epidemiology, natural history and registration of dystrophinopathies in the Netherlands".

Mutation analysis

DNA was extracted from whole blood taken from patients by a Gentra Puregene DNA purification Kit (Gentra Systems, Minneapolis, USA), following the manufacturer's instructions. Mutation analysis of the *DMD* gene was performed using multiplex ligation-dependent probe amplification (MLPA kit Salsa P034/P035 MRC-Holland, Amsterdam the Netherlands).

Cardiac analysis

Data were retrieved from a previous study, which had collected information on a 12-lead ECG and echocardiography of dystrophinopathy patients and female carriers.[177] The 12-lead ECG was screened for the following abnormalities (1) increased R-wave in V1 (>4mm), increased R-S ratio in V1 or V2 in the absence of a complete or incomplete right bundle branch block, or (2) pathological Q waves (>0.2 mV) in lateral (I, AVL, V6) or inferior leads (II, III, AVF), or (3) a complete or incomplete left bundle branch block or complete right bundle branch block.

Echocardiography was performed using a Vivid 5 GE echocardiograph equipped with a 5 MHz transducer, and measured Left Ventricle End Diastolic Diameter (LVEDD), and Left Ventricle End Systolic Diameter (LVESD), both in parasternal long axis projection. Global Left Ventricular Function (LVF) was judged as good, fair, or poor by an experienced cardiologist (LD). The Fractional Shortening Index (FSI) was calculated as follows: ((LVEDD-LVESD)/LVEDD)*100%. The LVEDD was corrected for weight and BSA.[178] Dilated cardiomyopathy (DCM) was defined as an enlarged left ventricle with a global left ventricle dysfunction or fractional shortening of 28% or less.[179]

For patients not involved in above mentioned study, cardiac data were collected from their treating cardiologist when possible. All ECGs were reassessed by an independent cardiologist (LD). For DMD patients, available echocardiography reports were gathered from treating cardiologists.

Western Blot analysis

A muscle biopsy from the anterior tibialis muscle was processed as previously described by van Deutekom et al.[140] Protein lysates were generated from muscle biopsies and Western blotting was performed according to previously described methods.[140,180] Monoclonal NCL-DYS1 (dilution 1:100, Novacastra, UK) or polyclonal ab15277 (dilution 1: 200 Abcam, UK) were used to detect dystrophin. Rabbit polyclonal antibody to sarcomeric alpha-actinin ab72592 (dilution 1/ 500 Abcam, UK) was used as a loading control. Blots were visualized and quantified with the Odyssey system and software (Li-Cor, USA) as described previously.[181,182] Samples obtained from the tibialis anterior muscle and medial and vastus lateralis muscles of five healthy males were used as reference samples. For each patient sample at least 2 technical replicates were performed. The average level of both dystrophin antibodies was noted.

Immunohistochemistry

Sections of 10 µm were cut from the anterior tibialis biopsies using a Shandon Cryotome (Thermo Fisher Scientific Co., Pittsburgh, PA, USA). Sections were fixed for 1 min with ice-cold acetone. Goat polyclonal dystrophin diluted 1:50 (SC-7461, Santa Cruz Biotechnology, USA) and rabbit polyclonal β-dystroglycan diluted 1:50 (SC-28535, Santa Cruz Biotechnology, USA) and rabbit polyclonal gamma-sarcoglycan diluted 1:50 (ab104478; Abcam, UK) were used to detect, dystrophin, beta-dystroglycan and gamma-sarcoglycan. Alexa-fluor 488 donkey-anti goat IgG (A11055, Invitrogen, the Netherlands) diluted 1:1000 and Alexa-fluor 594 donkey-anti rabbit IgG conjugate (A-21207, Invitrogen, the Netherlands) diluted 1:1000 were used as secondary antibodies. Slides were analysed using a fluorescence microscope (DM RA2; Leica Microsystems Wetzlar, Germany), and digital images were taken using a CCD camera (CTR MIC; Leica Microsystems). Staining of beta-dystroglycan and gamma-sarcoglycan was subjectively scored as absent, low (mainly cytoplasmatic staining), moderate (some (less than 50%) membrane bound fibers) or good (the majority of fibers have membrane bound staining).

Statistical analysis

Differences in disease course between our cohort and BMD patients with other mutations were analysed using the Kaplan Meier Survival Analysis. Statistical analysis was performed on these data through a Log Rank Test. The possible effect of dystrophin quantity on age at first symptoms was analysed using Pearson's Correlation. Differences in average age at first symptoms between patients having absent/low beta-dystroglycan or gamma-sarcoglycan staining and those having moderate/good staining were assessed using an independent T-test. All tests were performed at a significance level of 0.05.

Results

Participants

Out of the 120 BMD patients known in our registry, 48 met the inclusion criteria of the present study, representing 43 different families. Of the excluded 72 BMD patients, a mutation was known in 42. Their mutations were: point mutations (8), duplications (11), deletions not equaling a DMD mutation after skipping of one single exon (19) and out of frame mutations (4). The median age of participants was 43 years (range 6 to 67 years). Three participants had died at the time of the study at an age of 45 (stroke), 50 (DCM) and 76 years (cause of death unknown) respectively. Information about these patients was gathered through their family. The 48 patients had eight different in-frame deletions that could theoretically mimic the rescue of ten different out-of-frame deletions in DMD patients by the skip of one exon (Table 1). Thirty-two DMD patients representing six out-of-frame counterparts of these mutations were known in our national registry and were analysed for comparison.

Table 1. Frequency of "skipped" mutations per exon.

DMD mutation	Exon to skip	Resulting in-frame BMD mutation	Number of patients
deletion exon 10-21	22	deletion exon 10-22	1
deletion exon 30-43	44	deletion exon 30-44	2
deletion exon 46-47	45	deletion exon 45-47	25
deletion exon 46-48		deletion exon 45-48	8
deletion exon 46-49		deletion exon 45-49	4
deletion exon 46-53		deletion exon 45-53*	2
deletion exon 46-55		deletion exon 45-55*	5
deletion exon 50	51	deletion exon 50-51	1
deletion exon 45-52	53	deletion exon 45-53*	2
deletion exon 45-54	55	deletion exon 45-55*	5

* Mutation is mentioned twice, because it could be the result of skipping of two different exons.

Disease course

First symptoms occurred at a median age of 8 years (range 3 to 36). Most frequent first symptoms were sporting and walking difficulties, falling and myalgia (Table 2). Age at diagnosis ranged from 4 to 65 years (mean 19; median 16 years). Thirty-nine patients were ambulant, of whom four patients used a walking aid and twelve patients used a wheelchair intermittently. Nine patients were wheelchair dependent. The age at which these patients lost ambulation ranged from 26 to 56 years of age. None of the patients had scoliosis surgery. Use of ventilatory support was rare, with only one patient using night-time non-invasive mechanical ventilation from the age of 48 years. All clinical data are summarized in the supplementary data (Supplementary Table 1). The 48 BMD patients with the 'skipped" DMD mutations showed a later onset of first symptoms (mean 12 versus 8.0 years; p=0.054) and a significantly milder course of disease as measured by age at wheelchair dependence (estimated mean 53 versus 40 years; p=0.002) than the

Table 2. First symptoms for patients in our cohort as indicated by patients themselves.

	Frequency	Percentage
Sporting difficulties (running, cycling, swimming)	12	25
Walking difficulties (including waddling gait)	8	17
Falling	7	15
Difficulties walking stairs	7	15
Myalgia	7	15
Toe walking	7	15
Muscle cramps	6	13
Rising from chair	5	10
Muscle weakness	3	6
Fatigue/endurance	3	6
Large calves	2	4
Flat feet	1	2
Myoglobulinuria	1	2
Muscle wasting	1	2
Problems with lifting heavy objects	1	2
None	2	4
None mentioned	7	15

42 BMD patients with other DNA-proven mutations (Figure 2). When comparing our BMD patients with their 32 DMD "counterparts", all disease milestones (wheelchair dependence, ventilatory support and death) were reached at a later age in the BMD patients, showing the theoretical benefit of (single) exon skipping (Table 3). The groups were too small to perform statistical analysis.

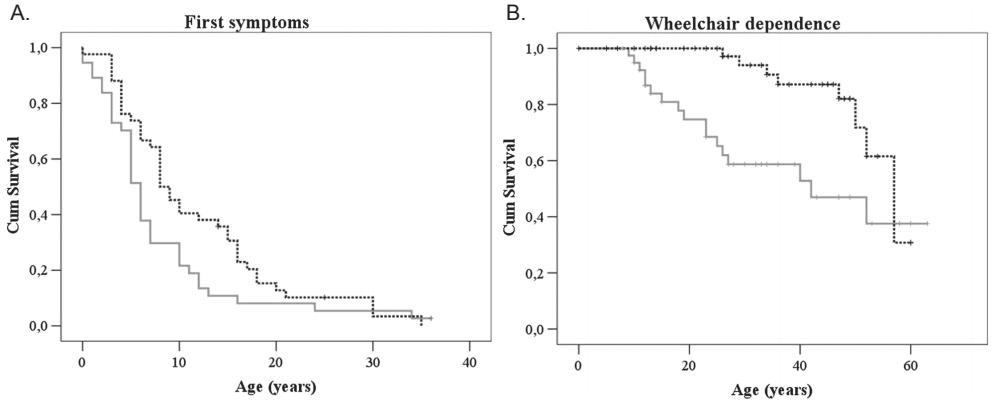

Figure 2. Clinical comparison of BMD study participants and other BMD patients

A. Survival analysis of age at first symptoms. The dotted line represents the survival curve for participants of the current study, in black all other other DNA-proven patients with BMD in the DDD.

B. Survival analysis of ambulatory status. The dotted line represents the survival curve for participants of the current study; in black all other DNA-proven patients with BMD in the DDD.

Table 3. Individual clinical comparison of BMD patients and their DMD mutation counterparts.

Exon to be skipped	Mutation	Average age (range)	Wheelchair use					Respiratory support			Death			Dilated cardiomyopathy				
			Always	*Partial*	*None*	*Average age wheelchair dependence (range)*	*Average age non wheelchair dependent patients (range)*	*Number*	*Average age start respiratory support (range)*	*Average age of patients not on respiratory support (range)*	*Number*	*Average age at death (range)*	*Average age of living patients (range)*	*Yes*	*No*	*Unknown*	*Average age at DCM (range)*	*Average age non DCM patients (range)*
53	DMD: del 45-52	14 (7-23)	7	0	1	8.4 (8-14)	7	2	10.5 (9-12)	16 (7-23)	2	14.5 (14-15)	11.8 (7-23)	2	3	3	13.0 (10-16)	13.0 (8-17)
	BMD: del 45-53	32 (15-49)	0	1	1	-	32 (15-49)	1	48	32 (15-49)	-	-	32 (15-49)	0	0	2	-	-
55	DMD: del 45-54	17 (7-38)	2	2	1	11.5 (10-13)	8.7 (7-11)	2	unknown	8.7 (8-11)	-	-	17.4 (7-38)	0	2	3	-	9.0 (9)
	BMD: del 45-55	47.4 (26-61)	0	0	5	-	47.4 (26-61)	-	-	47.4 (26-61)	-	-	47.4 (26-61)	0	3	2	-	32 (9-54)
45	DMD: del 46-47	15 (14-15)	1	1	0	7	15	1	8	15	1	14	15	0	0	2	-	-
	BMD: del 45-47	39.9 (11-63)	7	6	12	44.1 (26-57)	33.5 (11-63)	-	-	- (11-63)	-	-		7	12	6	40 (24-49)	33.9 (12-62)
	DMD: del 46-48	7	0	0	1	-	7	-	-	7	-	-	7	0	0	1	-	-
	BMD: del 45-48	44.9 (16-76)	1	3	4	50	40.4 (16-67)	-	-	44.9 (16-76)	-	-		3	5	0	50 (17-70)	30.8 (8-41)
	DMD: del 46-49	16 (6-25)	5	1	0	8.6 (5-13)	6	2	14.5 (14-15)	12.3 (6-18)	-	-	16 (6-25)	0	2	5	-	8.5 (6-11)
	BMD: del 45-49	23.8 (6-50)	1	2	1	29	21.3 (6-50)	-	-	23.8 (6-50)	-	-		0	2	2	-	15 (7-23)
51	DMD: del 50	11.5 (6-19)	5	5	0	10.2 (9-13)	8.4 (6-10)	1	16	10.7 (6-15)	1	1	11.5 (6-19)	1	3	6	11	11.0 (7-13)
	BMD: del 50-51	15	0	0	1	-	15	-	-	15	-	-	15	0	1	0	-	9

Education

Educational levels were similar to those of the general Dutch population and higher than those of Dutch DMD patients (Table 4). Thirty-eight out of 45 patients (84%) attended a regular primary school. Two patients attended a school for chronically disabled children, while five patients went to a school for children with behavioral difficulties. Six patients (15%) had to attend a secondary school for chronically disabled children.

Table 4. Highest level of education for patients aged 15 and older compared to DMD patients and the general Dutch population.

Highest level of education	Becker patients current study	General Dutch population[a]	Duchenne patients[b]
Practical Education	1 (2%)	9%	28 (19%)
Lower General Secondary Education	12 (28%)	24%	67 (45%)
Higher General Secondary Education/ Pre-University Education/Vocational Education	21 (49%)	41%	44 (29%)
University of Applied Sciences	6 (14%)	16%	8 (5%)
University	2 (5%)	9%	3 (2%)
Not mentioned	1 (2%)		

[a] data CBS Statline

[b] data retrieved from Dutch Dystrophinopathy Database

Cardiac features

Cardiac data were available for 36 patients. Twenty-nine patients had participated in the previous cardiac study. Cardiac analysis in these patients was performed at a median age of 31 years (range 8-55 years). For seven other patients (median age 40; range 7-70 years), recent cardiac data were retrieved from regular cardiac follow-up. Of twelve patients no cardiac data were included; four patients did not undergo any cardiac screening, while we were unable to retrieve cardiac data from the other eight.

ECG results were present for 32 patients (26 study participants/6 others), echocardiography results for all 36 patients, and Holter ECG was performed in 25 patients (21 study participants/4 others). Eight patients showed no abnormalities in all three examinations (median age at examination 31; range 8-55 years). None of the 25 patients who underwent Holter ECG showed ventricular tachycardia (VT), none-sustained VT, atrial-ventricular conduction defects or atrial fibrillation. Ten patients (28%) met the criteria for DCM. There was no correlation between the occurrence of cardiomyopathy and ambulation (Supplementary Table 1). Only three of the thirteen DMD patients for whom we were able to retrieve cardiac data met the criteria for a DCM (Table 3).

Muscle biopsy

Muscle biopsy data were present for 13 BMD patients with five different mutations. The average dystrophin level was 38% (range 7-71%) (Supplementary Table 1). Beta-dystroglycan staining revealed that in 6 patients beta-dystroglycan was primarily cytoplasmatic, while in 6 patients it was primarily membrane

bound in either some (3/13) or the majority (3/13) of muscle fibers. For one biopsy beta-dystroglycan staining was uninformative, as the material was too fibrotic. For gamma-sarcoglycan, staining was absent for one patient (exon 45-47 deletion), primarily cytoplasmatic for 2/13, and primarily membrane bound for 8/13 patients (in some fibers for 6/13 patients and in the majority of fibers for 2/13). Gamma-sarcoglycan staining could not be assessed for two biopsies due to the poor quality of the muscle tissue (fibrotic).

No correlation was found between dystrophin levels and age at first symptoms (R -0.037 p 0.90). Furthermore, no difference was observed between the average age of first disease symptoms for patients showing absent or low beta-dystroglycan or gamma-sarcoglycan staining compared to patients with moderate or good staining (beta-dystroglycan 18 versus 13% p 0.21; gamma-sarcoglycan 10 versus 16% p 0.38). The occurrence of other disease milestones was too low to perform a meaningful statistical analysis.

Discussion

Our study suggests that successful skipping of exons 22, 44, 45, 51, 53 or 55 in patients with DMD would significantly ameliorate the course of the disease. All 48 BMD patients with various in-frame deletions showed a disease course that was milder than that of patients with DMD, in terms of cognitive function, mobility, cardiac and respiratory function, as shown by comparison with DMD patients from our national registry as well as with the DMD disease course as known from literature. Remarkably, the milder disease course was further underlined by the percentage of patients that was wheelchair dependent in this subset of patients when compared to the 42 BMD patients with other mutations (i.e. deletions other than of exons 10-33, 30-44, 45-47, 45-48, 45-49, 45-53, 45-55 or 50-51). This might be explained by the localization of the mutations within the *DMD* gene and highlights the importance of investigating each subgroup of BMD patients with a mutation equaling a DMD mutation after successful exon skipping separately.

Two previous studies reported a mild disease course in 19 BMD patients with in-frame deletions ending with exon 51, 53 and (multi exon skip) 55.[104,183] Our data support these results and in addition provide information about the phenotypes of BMD patients with deletions ending with four other exons (22, 44, 45, and 46). These results are relevant for a large proportion of DMD patients (an estimated 13%, 8%, 2%, 0.6%, 6% and 8% for exon 51, 53, 55, 22, 44 and 45 respectively).[170]

In our BMD cohort all clinical disease parameters were delayed compared to DMD patients. In DMD patients first symptoms are clearly present at the age of four, while first symptoms in our BMD patients were only noticed at an median age of eight years.[184] The progression of ambulatory difficulties is significantly slower, with the youngest BMD patient becoming wheelchair dependent at 26 years, while almost all DMD patients have lost ambulation when reaching puberty.[8]

Another milestone in disease progression for DMD is mechanical ventilation, with pulmonary failure being a major cause of death. However, the introduction of respiratory support has significantly improved

survival, from an average survival in the teenage years to the twenties and thirties.[7,8,14] While the average DMD patient becomes ventilator dependent before the age of twenty, only one of the BMD patients in our study used non-invasive night-time ventilatory support, starting at the age of 48 years.[8,14]

Mortality in DMD is also often caused by cardiac failure.[7] We found DCM in 28% of the BMD patients, which is consistent with previous studies, in which DCM was observed in 17 and 32%, respectively.[19,185] Our study showed ECG changes in 13 out of 32 patients (41%). Previous studies found ECG abnormalities in 45 to 89% of BMD patients,[31,186,187] but the small size of the populations in all studies (<30 patients) and the different ages at cardiac examination, could have biased the results. A study in 328 DMD patients by Nigro et al showed echocardiographic signs of a dilated cardiomyopathy in 45% of DMD patients between 14 and 18 years.[188] In the adult DMD population they found signs of cardiomyopathy or conduction defects in all patients, with 72% meeting the criteria for DCM. The present study showed a DCM in only 7/29 (24%) adult BMD patients. Unfortunately, no data about age at presentation of DCM were available for these patients.

Although the delivery of antisense oligonucleotides (AON) to the heart for exon skipping technology is more difficult than to skeletal muscle, current developments show expression of the exon skipping therapeutics in murine heart muscle.[189-191] Though part of the difference in prevalence of DCM between our BMD study and that of DMD patients by Nigro et al could be explained by a difference in definition of DCM, our data feature an important possible benefit of exon skipping in DMD patients.

AON delivery to brain is another concern as systemically delivered AONs do not cross the blood brain barrier.[192] Since DMD can be accompanied by cognitive impairment, brain expression of dystrophin is important.[193] Notably, it has been shown that upon intraventricular treatment of adult mice, exon skipping mediated dystrophin restoration resulted in correction of neuronal plasticity and behavior, suggesting that part of the cognitive impairment could be reversible.[194,195] The patients presented in the current study provide a distribution of educational level similar to the general Dutch population, showing no signs of intellectual deficits. However, in previous studies intellectual deficits in BMD patients have been described.[21,196] This difference could signify clinical differences between BMD mutations, but could also be caused by our relatively small sample size.

Ten percent of our patients attended a school for children with behavioral difficulties. This percentage is higher than the prevalence in the general population, but lower than the prevalence of neuropsychiatric disorders (ADHD, autism and obsessive-compulsive disorders) in DMD patients (14%), although a strict comparison is difficult, since the level of impairments caused by these neuropsychiatric disorders in DMD patients is unknown.[197] If the concerns of AON delivery to the brain could be overcome, the differences in cognitive functioning and occurrence of neuropsychiatric disorders could constitute another possible improvement in the disease course of DMD patients.

Finally, the milder disease progression in our BMD cohort compared to DMD patients concerns survival. In our cohort the median age is 43 years, with 11 patients being older than 50 years. Two patients had died at the relatively young age of 45 and 50 years respectively. In DMD, however, the average survival is within the 30s age range.[7,8]

In our study we did not find a significant correlation between dystrophin levels or beta-dystroglycan/gamma-sarcoglycan expression and disease severity as measured by age at first symptoms. However, our data concerning muscle biopsies was limited to a group of only 13 patients representing five different mutations.

Our data show the possible result of exon skipping in DMD when achieved from very young or even conceptual age, and therefore represents an optimistic viewpoint. The outcome might be less favorable if exon skipping becomes a clinical reality. Since the average age at diagnosis is 4.5 years, the potentially positive effects of exon skipping treatment may be less than described here.[161] A systematic neonatal CK screening for DMD might be warranted, to enable starting therapy as early as possible. Aside from the diagnostic delay, unforeseen technical problems might impair an optimal treatment with AON, possibly due to the less efficient exon skipping in some tissues like the heart, or the brain.

One could argue that our BMD cohort has been biased towards the selection of less affected patients. However, as our database covers over 50% of all Dutch BMD patients, and patients were recruited using multiple sources, like patient organizations, neurologists, rehabilitation specialists, centers for ventilatory support, genetic diagnostic databases and the internet, a selection bias is unlikely. This is supported by the fact that the clinical characteristics of our BMD cohort in the DDD were similar to another study on a large number of BMD patients, with a similar frequency of and age at wheelchair dependence.[21] Within our study cohort patients with cardiac data did not differ from the other patients, when compared on age, age at first symptoms, use of a walking aid and wheelchair dependence.

In conclusion, our report strongly supports the potential benefit of exon skipping treatment for DMD. Compared to DMD patients, BMD patients with an in-frame deletion equalling the mutation achieved after successful exon skipping of an out-of-frame DMD deletion have a milder disease course, as can be concluded from data concerning ambulation, education, pulmonary and cardiac function and survival. Hopefully the outcome of exon skipping trials will support these results and indeed show clinically significant improvements in the health and physical function of patients with DMD.

Supplementary Table 1. Overview of individual clinical and muscle biopsy data of all 48 participating BMD patients.

Mutation	Age	Muscle biopsy			Age first symptoms	Ambulation			Respiratory support	Cardiac examination		
		Dystrophin level (%)	*Beta-dystroglycan*	*Gamma-sarcoglycan*		*Use of walking aid*	*Wheel-chair use*	*Age wheelchair dependence*		*Age examination*	*ECG*	*Echo cardi-ography*
Del10-22	46	50	Good	Good	14	Yes	None		No	40	-	NA
Del 30-44	23	7	Low	Low	5	No	None		No	15	NA	NA
Del30-44	14				4	No	None		No	14	NA	NA
Del45-47	47				18	Yes	Partially		No	40	NA	DCM
Del45-47	62				7	-	Always	57	No	62	Complete RBBB, R/S>1 in V2	NA
Del45-47	46				20	No	None		No			
Del45-47	62	20	Low	Moderate	30	-	Always	57	No	55	NA	NA
Del45-47	38	71	Low	Absent	21	No	None		No	31	NA	NA
Del45-47	56				35	Yes	Partially		No	49	Q in aVL	DCM
Del45-47	27				9	No	None		No	24	Incomplete RBTB	DCM
Del45-47	56				18		Always	52	No	50	Complete RBBB	NA
Del45-47	28				3	No	None		No	27	R/S>1 in V2	NA
Del45-47	50	38	Moderate	Moderate	16	Yes	None		No	43*	NA	DCM
Del45-47	63				6		Always	26	No			
Del45-47	35	38	Moderate	Moderate	15	Yes	Partially		No			
Del45-47	11				3	No	None		No			
Del45-47	13				8	No	None		No			
Del45-47	50†				6	Yes	Partially		No	42*	NA	Death due to DCM
Del45-47	45	18	Good	Moderate	16	-	Always	36	No	38	NA	DCM
Del45-47	39				9	-	Always	34	No	31	R in V1>4mm	NA
Del45-47	28	64	Good	Moderate	12	No	None		No	23	NA	NA

Supplementary Table 1. Overview of individual clinical and muscle biopsy data of all 48 participating BMD patients. (continued)

Mutation	Age	Muscle biopsy			Age first symptoms	Ambulation			Respiratory support	Cardiac examination		
		Dystrophin level (%)	*Beta-dystroglycan*	*Gamma-sarcoglycan*		*Use of walking aid*	*Wheel-chair use*	*Age wheelchair dependence*		*Age examination*	*ECG*	*Echo cardi-ography*
Del45-47	45†				10	No	Partially		No	44	R in V1>4mm, Q in I and aVL	DCM
Del45-47	33				unknown	No	None		No	32	-	Slightly reduced LVF
Del45-47	19	38	Moderate	Low	4	No	None		No	12	Incomplete RBBB, R in V1>4mm, R/S>1 in V2	NA
Del45-47	25	17	Low	Moderate	21	No	None		No	19	-	NA
Del45-47	38	48	Low	Undetermined	17	Yes	None		No	31	NA	NA
Del45-47	42				8	Yes	Partially		No	34	NA	NA
Del45-47	54				4	-	Always	47	No			
Del45-48	16				unknown	No	None		No	8	Incomplete RBBB	NA
Del45-48	47	34	Low	Undetermined	15	Yes	None		No	40	NA	FSI 13%, slightly reduced LVF, normal left ventricle diameter
Del45-48	48				10	Yes	Partially		No	40	NA	NA
Del45-48	25				4	No	None		No	17	R in V1>4mm, Q in I and aVL	DCM
Del45-48	32				3	No	Partially		No	25	NA	Slightly reduced LVF
Del45-48	67				30	No	Partially		No	62	Complete LBBB	DCM
Del45-48	48				16	No	None		No	41	-	NA
Del45-48	76†				6	-	Always	50	No	70	NA	DCM

Supplementary Table 1. Overview of individual clinical and muscle biopsy data of all 48 participating BMD patients. (continued)

Mutation	Age	Muscle biopsy			Age first symptoms	Ambulation			Respiratory support	Cardiac examination		
		Dystrophin level (%)	*Beta-dystroglycan*	*Gamma-sarcoglycan*		*Use of walking aid*	*Wheel-chair use*	*Age wheelchair dependence*		*Age examination*	*ECG*	*Echo cardi-ography*
Del45-49	31				8	-	Always	29	No	23	NA	Borderline dilated left ventricle
Del45-49	8				3	No	None		No	7	R>4mm in V1, R/S>1 in V2	NA
Del45-49	50				36	No	Partially		No			
Del45-49	6				unknown	No	Partially		No			
Del45-53	15				8	No	None		No			
Del45-53	49				8	Yes	Partially		Yes, at age 48			
Del45-55	61				8	No	None		No	54	NA	NA
Del45-55	50	47	Undeter-mined	Good	6	No	None		No			
Del45-55	53				8	No	None		No	46	NA	NA
Del45-55	47				12	No	None		No			
Del45-55	26				4	No	None		No	19	R>4mm in V1	Borderline dilated left ventricle
Del50-51	15				unknown	No	none		No	9	NA	NA

NA: no abnormalities; PVC: premature ventricular contraction; DCM: dilated cardiomyopathy; VES: ventricular extrasystole; LBBB: left bundle branch block; RBBB: right bundle branch block; FSI: fractional shortening index; LVF: left ventricular function

† Age at time of death