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Prolonged ambulation in Duchenne patients with a mutation amenable to exon 44 skipping

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Abstract

Duchenne muscular dystrophy has a severe disease course, though variability exists. Case reports suggest a milder disease course of patients amenable to exon 44 skipping. In this study, we analysed this and show that age at wheelchair dependence in patients with a dystrophin deletion requiring exon 44 skipping is postponed compared to patients with a deletion skippable by exon 45, 51 and 53 (10.8 versus 9.8 years; *P* 0.020). This may be explained by more frequent spontaneous exon 44 skipping in patients with a deletion flanking exon 44. This finding has important implications for the development of future Duchenne trials.

Introduction

Duchenne and Becker Muscular Dystrophy (DMD/BMD) are X-linked muscle diseases, caused by mutations in the *DMD* gene, coding for the muscle membrane-stabilizing dystrophin protein. DMD is generally caused by out-of-frame mutations in the *DMD* gene, leading to the absence of dystrophin, while in-frame mutations allow production of internally deleted or duplicated but functional dystrophin proteins and are associated with the less severe BMD. However, there are some exceptions. First, mutations at the 5' end of the *DMD* gene often violate the reading-frame rule, as there are many examples of BMD patients with out-of-frame mutations. This inconsistency is generally thought to be caused by the activation of alternative translation initiation sites and alternative splicing.[162,166-168] Secondly, there are several reports describing patients with a deletion of exon 45 (out-of-frame) and a BMD or intermediate phenotype.[92,93] This could result from low level spontaneous exon 44 skipping, which would restore the reading frame. Most reports of exon 45 deleted patients doing better than expected are anecdotal. A recent study by Pane et al showed a trend towards a slower decline in the 6MWT over a period of one year in exon 44 skippable patients compared to patients with other skippable mutations.[169] To study this in more detail we have compared the age at wheelchair dependence of DMD patients with out-of-frame mutations who would benefit from skipping of exon 44 to patients who would benefit from skipping of the three other most prevalent skippable exons (45, 51 and 53) in a large cohort of 107 DMD patients.[170]

Patients and methods

Patients were recruited from the Dutch Dystrophinopathy Database, which contains clinical and DNA information about 363 DNA-confirmed DMD patients. Patients were included if they had an out-of-frame mutation for which the reading frame would be restored by skipping of exons 44, 45, 51 or 53 respectively. Age at wheelchair dependence and steroid use were recorded. Steroid treatment was defined as "using steroids for one year or more prior to loss of ambulation". This limit was chosen to ensure there was sufficient time for the steroid treatment to influence the age at wheelchair dependence. Patients who started less than one year prior to loss of ambulation or who started less than one year ago and were still ambulant were excluded.

A survival analysis for loss of ambulation was performed using Cox Regression Analysis, with exon 44 skippable or 45, 51 and 53 skippable and steroid use as covariates. As the patients with a mutation skippable by exon 45, 51 and 53 show similar results, these data were pooled. Our hypothesis included a loss of ambulation at a later age for the exon 44 skippable patients, therefore tests were performed using a one-sided *T*-test, at a significance level of 0.05. Differences in frequency of steroid users and steroid regimen between the two different groups were analysed using the Chi Square test.

Results

One-hundred-and-twenty-seven patients with a mutation skippable by either exon 44, 45, 51 or 53 were registered. Thirteen patients were excluded due to unknown steroid status. This led to the inclusion of

Table 1. Estimated age at wheelchair dependence for patients with different skippable mutations.

Exon to be skipped	Steroid treated patients		Non-steroid treated patients	
	n	*Age (SE)*	*n*	*Age (SE)*
Exon 44	11	13.0 (0.97)	22	10.2 (0.40)
- del 19-43	1			
- del 23-43	1			
- del 35-43	1			
- del 43			4	
- del 45	6		16	
- del 45-54	2		2	
Exon 45	9	11.4 (0.91)	10	8.6 (0.56)
- del 12-44	2			
- del 44			2	
- del 46			1	
- del 46-47	1		2	
- del 46-49	4		1	
- del 46-51	2		4	
Exon 51	15	11.0 (0.65)	17	9.4 (0.52)
- del 45-50	2		6	
- del 48-50	3		4	
- del 49-50	1		3	
- del 50	8		2	
- del 52	1		2	
Exon 53	13	10.2 (0.52)	17	8.7 (0.53)
- del 45-52	4		5	
- del 48-52	4		3	
- del 49-52	4		6	
- del 50-52	1		3	
Overall	48	11.3 (0.44)	66	9.4 (0.26)

Data are presented as mean (standard error) for patients amenable for exon 44, 45, 51 and 53 skip respectively. The numbers of patients are subdivided into the different presented mutations for each skippable mutation individually.

33 patients with an exon 44 skippable mutation in the study and 81 controls (19 skippable by exon 45, 32 by exon 51 and 30 by exon 53; Table 1). Thirty-three patients were still ambulant at the time of the study (10, 7, 11 and 5 for the exon 44, 45, 51 and 53 skippable mutations respectively). Forty-eight patients (42%) used steroids, of whom 40 (83%) followed a 10 days on/10 days off or 10 days on/20 days off regimen. There was no significant difference in frequency of steroid users or steroid regimen between the exon 44 skippables versus the others (*P 0.23* and *P 0.72)*. The age at loss of ambulation was significantly higher for the 33 patients with an exon 44 skippable mutation than for the 81 patients with other skippable mutations (10.8 versus 9.8 years; *P* 0.020) (Table 1 & Figure 1). There was also a significant effect of steroid use on age at wheelchair dependence (*P* 0.001).

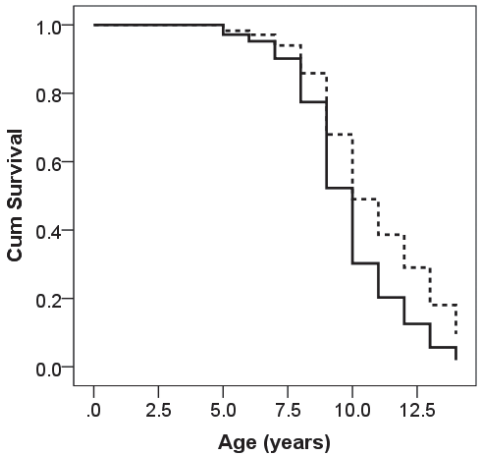

Figure 1. Survival analysis of age at wheelchair dependence
Data are shown for patients with a mutation eligible to skipping of exon 44 (dotted line), compared to the exons 45, 51 and 53 (black line).

Discussion

Our results confirm that patients with a deletion amenable to exon 44 skipping have a less severe disease course than patients with 45, 51 or 53 skippable mutations. For patients with an exon 45 deletion on DNA level, but a disease course more consistent with BMD, low level of spontaneous exon 44 skipping has been reported previously.[92,93,171,172] This restores the reading frame, allowing the production of some dystrophin. The finding that exon 44 skippable patients have a milder disease course suggests that spontaneous skipping of exon 44 occurs frequently, although the present study lacks data from dystrophin RNA and dystrophin expression studies.

Interestingly, a positive effect of corticosteroid use on ambulation was seen for all four different groups of patients. The potential low expression of dystrophin in the exon 44 skippable patient group does not clearly change the beneficial effect of corticosteroids.

The effect of mutation on disease course could have implications for future trials. As patients with an exon 44 skippable mutation show a slower pace of disease progression, stratification for mutation should be considered when planning trials for drugs that are not mutation specific. Secondly, caution should be used when 'sharing' placebo groups for different exon skipping trials. As patients with an exon 44 skippable mutation show a less severe disease course than other patients, they cannot be used as placebo group for e.g. exon 53 skipping trials, nor the other way around.

This study has some limitations. Firstly, we here suggest that the possible differences in disease course are caused by spontaneous skipping of exon 44. However, we did not perform a muscle biopsy to investigate dystrophin RNA and dystrophin expression to validate this hypothesis. Material from previous biopsies was not available, and performing a new muscle biopsy in the boys for this study was impractical for obvious medical and ethical reasons. A second limitation is the retrospective nature of our study.

However, recent studies by Pane et al and Servais et al (unpublished data) including prospective data also showed differences in disease severity between patients with different skippable mutations.[169]
In conclusion, in this study, we present data that suggest a less severe disease course for DMD patients with a deletion eligible for exon 44 skipping. Larger, more detailed studies are necessary to investigate this relationship in more detail and to further look into the physiological mechanism of this difference.

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