ARTICLE

The relationship between deficit in digit span and genotype in nonsense mutation Duchenne muscular dystrophy

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Abstract

Objective

To evaluate the relationship between deficit in digit span and genotype in nonsense mutation (nm) Duchenne muscular dystrophy (DMD) (nmDMD).

Methods

We investigated the relationship between normalized digit-span forward (d-sf) and digit-span backward (d-sb) scores to the location of nmDMD mutations in 169 participants ≥5 to ≤20 years who participated in a phase 2b clinical trial. Because alternative promoters are found upstream of *DMD* exons 30, 45, and 63, we correlated d-sf and d-sb to the specific nmDMD mutation location.

Results

Participants with nm downstream of exon 30, downstream of exon 45, and downstream of exon 63 had significantly lower normalized d-sf scores ($p < 0.0001$). Participants with nm downstream of exon 45 in addition had significantly lower normalized d-sb score ($p < 0.04$). There was no significant difference in the normalized d-sb score in participants with mutations upstream or downstream of *DMD* exon 30 or upstream or downstream of *DMD* exon 63.

Conclusion

Our data provide evidence that specific cognitive deficits correlate to genotype in individuals with nmDMD, highlighting the critical role of brain-specific dystrophin isoforms in the neurobiological manifestations of this disease.

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Coinvestigators are listed at links.lww.com/WNL/A681.

Glossary

d-sb = digit-span backward; **d-sf** = digit-span forward; **DMD** = Duchenne muscular dystrophy; **nm** = nonsense mutation; **WM** = working memory.

Duchenne muscular dystrophy (DMD) is the most common monogenic disorder caused by mutations in the dystrophin gene (*DMD*) located on the X-chromosome. Approximately 15% of DMD are due to a nonsense mutation (nm) that prevents translation of dystrophin mRNA into a functional protein.[1] In addition to progressive skeletal and cardiac muscle manifestations, the higher incidence of attention-deficit/hyperactivity disorder, obsessive-compulsive disorder, and autism spectrum disorder posits the critical importance of dystrophin for brain development.[2] The neurobiological manifestations in DMD are in part due to the loss of full-length dystrophin (dp427) and truncated dystrophin isoforms (dp260, dp140, and dp71) in the brain. These isoforms derive from alternative brain-specific promoters that use specific exons as the N-terminal domain and are as follows: exon 1 for dp427, exon 30 for dp260, exon 45 for dp140, and exon 63 for dp71. In nmDMD, based on the location of the mutation, full-length and truncated dystrophin will be absent.

A relationship between cognitive deficits and *DMD* genotype has been reported,[3,4] with the lowest IQ in those with mutations distal to exon 63[5] and those with point mutations.[6] A normal IQ can be misleading in DMD[7]; regardless of IQ, poor performance on digit span—a task that specifically tests working memory (WM)—is notable in this condition.[8] Thus, WM deficit is a characteristic neuropsychological profile in DMD. The pervasive importance of WM in intellectual ability is because of its effect on attention regulation and executive function. As previous studies of cognition in DMD have focused exclusively on correlating IQ with *DMD* genotype, there is a gap in our knowledge regarding the relationship between digit span and *DMD* genotype.

Our primary objective was to evaluate the relationship between deficit in digit span and *DMD* genotype. We evaluated this hypothesis by correlating digit-span forward (d-sf) and digit-span backward (d-sb) to *DMD* genotype in 169 participants with nmDMD. Participants with mutations downstream of exon 45 have significantly lower normalized median d-sf and d-sb scores. Our data provide scientific evidence for a clear correlation between deficit in digit span and *DMD* genotype in nmDMD.

Methods

The data presented here were obtained from participants at their baseline study evaluation prior to enrollment in a prospective, phase 2b clinical trial.[9]

Standard protocol approvals, registrations, and patient consents

Institutional review boards/ethics committees approved the study protocol. The study was conducted in accordance with the Declaration of Helsinki (2000) and the principles of Good Clinical Practice according to the International Conference on Harmonization. This study is registered under Clinical trials.gov (NCT02090959).

Study participants

Ambulatory boys inclusive of ages 5–20 years with nmDMD diagnosis confirmed by gene sequencing were enrolled. Participants had been on stable dose of oral glucocorticoids for at least 6 months prior to study enrollment. All participants provided written consent to participate.

Study measures

d-sf and d-sb, which measure WM capacity and WM load, respectively, were assessed by a trained study team member. The tasks were performed as follows. A series of digits (0–9) were presented to the participant in an auditory format only. To evaluate both d-sf and d-sb, 3 digits were presented at the rate of 1 digit per second. The same digits were presented to all participants.

To assess d-sf, the participant was requested to repeat back the digits in the order they were presented; to assess d-sb, the participant was requested to reverse the order of presentation. A raw score of the total number of correct responses was converted to an age-scaled score using the appropriate normative values for the child's age and language.[10]

nmDMD mutation locus and participant grouping

We evaluated each participant based on individual nmDMD location and categorized participants into 2 groups based on whether their nm was located upstream or downstream of exon 30, upstream or downstream of exon 45, or upstream or downstream of exon 63.

Statistical analysis

A Wilcoxon rank-sum test was used to compare median normalized score between nmDMD locations. The correlation between digit span and *DMD* genotype was analyzed using linear regression with SAS software version 9.4 (SAS Institute, Cary, NC). All analyses were 2-sided, and the level of statistical significance was set at 0.05.

Data availability

Individual de-identified participant data (including related documents such as study protocols, statistical analysis plans,

and data dictionaries) from this study, which was conducted between 2004 and 2010, will not be made available.

Results

The study enrolled 174 participants. The normalized d-sf was available in 170 participants, and d-sb was available in 169 participants. The median normalized d-sf score was 3 (range 1–15), and d-sb score was 1 (range 1–4). The mean age of study participants was 8.5 years (range 5–20 years; SD 2.6).

Comparison of digit span scores and nmDMD

Participants with nm downstream of exon 30, downstream of exon 45, and downstream of exon 63 had significantly lower normalized d-sf scores ($p < 0.0001$) (table, upper panel). Participants with nm downstream of exon 45 also had significantly lower normalized d-sb ($p < 0.04$) score. There was no statistically significant difference in the normalized d-sb score in those with mutations upstream or downstream of *DMD* exon 30, or upstream or downstream of *DMD* exon 63 (table, lower panel).

Correlation between digit span scores and nm location

Digit span scores were correlated to the location of nm. There was a negative correlation between the normalized d-sf score based on site of nm (figure, A). By contrast, there was no correlation between normalized d-sb score and nm either upstream or downstream of exon 45 and exon 63, but there was a correlation with nm downstream of exon 30 (figure, B).

Discussion

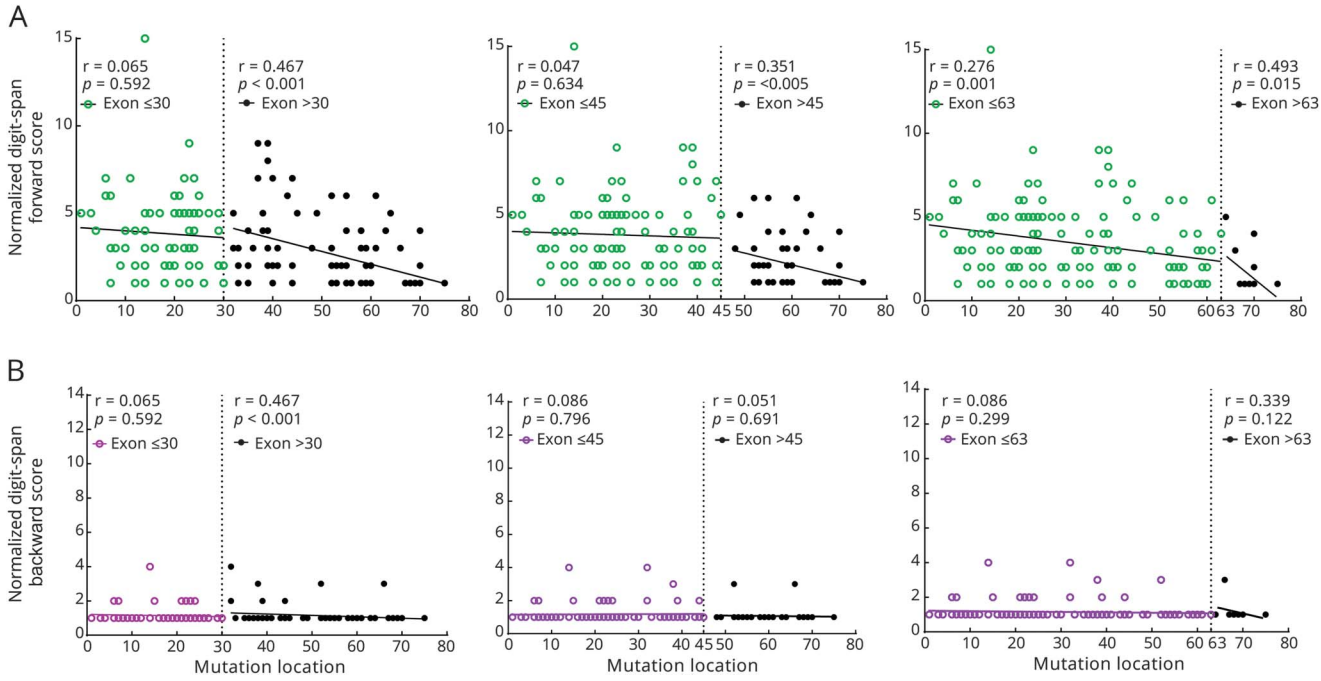
Using a large cohort of participants with nmDMD, we provide evidence, for the first time to our knowledge, of the relationship between deficit in digit span and nmDMD, with those individuals with nm located downstream of exon 45 having deficits in WM load and capacity. The rationale to focus on digit span is that robust deficit in this measure is detected in individuals with DMD regardless of the level of intellectual capacity. Our finding that individuals with nm downstream of exon 45 have abnormalities in digit span—a specific measure of WM—supports the role of dystrophin isoforms in brain development in DMD. Consistent with existent literature, those with DMD mutations that affect dystrophin dp140 isoform have prominent cognitive deficits.[4,11] The absence of brain-specific dystrophin isoforms not only has a functional consequence on cognitive skills, but also affects brain development. Smaller total and gray matter volume, and altered white matter microstructure, have been detected in individuals with mutations downstream of *DMD* exon 45.[11] The loss of multiple brain-specific dystrophin

Table Normalized digit-span forward (d-sf) (n = 170) and digit-span backward (d-sb) scores (n = 169) in participants with Duchenne muscular dystrophy

	No.	Median	Min	Max	*p* Value[a]
Normalized d-sf					
All	170	3	1	15	
Exon ≤30	71	4	1	15	<0.0001
Exon >30	99	2	1	9	
Exon ≤45	107	4	1	15	<0.0001
Exon >45	63	1	1	6	
Exon ≤63	146	3	1	15	<0.0001
Exon >63	24	1	1	5	
Normalized d-sb					
All	169	1	1	4	
Exon ≤30	71	1	1	4	0.497
Exon >30	98	1	1	4	
Exon ≤45	107	1	1	4	0.041
Exon >45	62	1	1	3	
Exon ≤63	146	1	1	4	0.197
Exon >63	23	1	1	3	

[a] Wilcoxon rank-sum test.

Figure Normalized digit-span forward (d-sf) score, normalized digit-span backward (d-sb) score, and Duchenne muscular dystrophy (DMD) mutation location



(A) Normalized d-sf score. (B) Normalized d-sb score.

isoforms in individuals with DMD may cumulatively increase the burden of cognitive deficits.[12] Our data suggest that specific mutations in nmDMD may disrupt the physiologic development of neural networks, which may, in turn, affect WM and influence cognitive reserve.

There is probably a broader role for dystrophin in brain development and function. In a postmortem analysis of 13 brains in DMD, dendritic length and branching were abnormal, which suggests that dystrophin may be important for normal neuronal morphology.[13] Glucose hypometabolism is seen in the cerebellum and temporal lobe as detected by PET imaging in individuals with DMD, supporting a functional role for dystrophin in normal brain function.[14] Understanding the molecular basis of these changes will allow for pharmacologic approach to improve cognition in DMD.

Some limitations of our study include the cross-sectional evaluation of WM, which does not permit evaluation of intellectual gains (or decline) over time in this population. Second, we did not perform comprehensive psychometric testing in our participants. Acknowledging these limitations, our data lend support for a correlation between digit span and nmDMD. WM predicts early school achievements in mathematics, reading, and writing in children.[15]

This study also highlights the necessity to reappraise the unmet needs of these individuals. Current therapeutic focus in DMD continues to be exclusively centered on restoring motor function. As new therapeutic agents have been approved recently for DMD, and life expectancy has increased in this population, we need to address strategies to help these individuals meet their self-efficacy milestones of adulthood. Although fundamental questions regarding the role of dystrophin in synaptogenesis and in activity-dependent myelination are yet to be understood, the improved understanding of the neurobiology of cognitive deficits in DMD offers an opportunity to readdress pragmatic strategies towards cognitive rehabilitation in affected individuals.

Author contributions

M. Thangarajh and G.L. Elfring analyzed and interpreted the data. M. Thangarajh prepared the manuscript. All authors contributed to revising the manuscript.

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Disclosure

M. Thangarajh has provided consultation services to PTC Therapeutics, Inc. G. Elfring is a full employee of PTC Therapeutics, Inc. P. Trifillis is a full employee of PTC Therapeutics, Inc. J. McIntosh is a full employee of PTC Therapeutics, Inc. S. Peltz is a full employee of PTC Therapeutics, Inc. Go to Neurology.org/N for full disclosures.

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