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Research Report

Proton Magnetic Resonance Spectroscopy Indicates Preserved Cerebral Biochemical Composition in Duchenne Muscular Dystrophy Patients

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Abstract.
Background: Duchenne muscular dystrophy (DMD) is caused by the absence of dystrophin. DMD is associated with specific learning and behavioural disabilities. In the brain, dystrophin is associated with $GABA_A$ receptors and aquaporin-4 in neurons and astrocytes, respectively, but little is known about its function.
Objective and Methods: In this study we aimed to compare the biochemical composition between patients and healthy controls in brain regions that are naturally rich in dystrophin using magnetic resonance spectroscopy. Given previous conflicting results obtained at clinical field strengths, we obtained data using a 7 Tesla system with associated higher signal-to-noise ratio and spectral resolution.
Results: Results indicated unchanged biochemical composition in all regions investigated, and increased variance in glutamate in the frontal cortex.

Keywords: Duchenne muscular dystrophy, brain, magnetic resonance spectroscopy, metabolites

INTRODUCTION

Duchenne muscular dystrophy (DMD) is an X-linked recessive disease caused by loss of dystrophin expression due to mutations in the *DMD* gene. DMD has a specific neurocognitive profile in that the mean full-scale intelligence quotient (FSIQ) is approximately one standard deviation below the population mean [1]. DMD patients exhibit problems with verbal short term memory, visuospatial long term memory and verbal fluency [2]. A higher incidence of autism spectrum disorders (ASD), attention deficit and hyperactivity disorders (ADHD), epilepsy and obsessive-compulsive disorders (OCD) occurs, and specific learning disorders such as dyslexia have been

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described [3–7]. Patients with distal mutations in the *DMD* gene present cognitive deficits more frequently than patients with proximal mutations [3, 8, 9], providing a link between the absence of multiple brain isoforms to increased risk of cognitive problems.

Dystrophin is naturally expressed in muscle cells, endothelial cells and the central nervous system [10] in various isoforms (i.e. Dp427, Dp260, Dp140, Dp116, Dp71 and Dp40). In the brain, dystrophin is believed to be expressed in the cerebral cortex, amygdala, hippocampus and in purkinje cells of the cerebellum [11–15]. Proximal mutations in the *DMD* gene only affect Dp427 in the brain, whereas distal mutations additionally affect Dp140 and Dp71. Little is known about the function of dystrophin in the brain but studies indicate that Dp427 is involved in the organization of $GABA_A$ receptors ($GABA_A$Rs) and Dp71 in aquaporin-4 (AQP4)-containing protein complexes in neurons and glia, respectively [10].

Using quantitative magnetic resonance imaging (MRI) we have previously shown reduced grey matter volume, altered white matter microstructure and reduced cerebral perfusion in DMD patients compared to age-matched healthy controls [16, 17]. Both structural and perfusion differences were measured throughout the brain and were more pronounced in patients with mutations predicted to affect Dp140 in addition to Dp427 expression. Because dystrophin is linked to $GABA_A$Rs and AQP4, it is also possible that the biochemical steady-state is altered in the absence of dystrophin. Previous magnetic resonance spectroscopy (MRS) studies looking at biochemical composition in the brain using clinical magnets have presented conflicting results, namely both increased and decreased choline, decreased glutamate and increased NAA [18–20]. In this study, taking advantage of a 7 Tesla system with higher signal-to-noise ratio and spectral resolution, we aimed to assess the steady-state biochemical composition of the brain regions known to express dystrophin normally.

METHODS

Twenty boys (8–18 years of age) with a mutation in the *DMD* gene were recruited from the Dutch Dystrophinopathy Database [21]. Seventeen healthy age-matched boys, without muscle or brain disorders, were recruited from local schools/leisure clubs using flyers. Exclusion criteria were the inability to lie supine for 45 minutes, or MRI contraindications. Patients receiving corticosteroid treatment according to a non-continuous schedule were scanned during the off-period. Both ambulant and non-ambulant patients were included. Written informed consent was obtained from participants and their parents/guardian: the study was approval by the local medical ethical committee.

Participants were scanned on a Philips Achieva 7T using a 16- or 32-channel Nova Medical receive head coil. A dielectric pad was placed at the lower back of the head [22]. Scan parameters are listed in Supplementary Table 1. A whole-brain 3D T1w-scan was acquired for anatomical reference. A B_0 map was acquired for first- and second-order shimming. Proton (^{1}H) MRS used a stimulated echo acquisition mode (STEAM) sequence preceded by variable power optimization relaxation delays (VAPOR) water suppression [23]. Volumes-of-interest (VOIs) were planned in the left hemisphere (Fig. 1). A non-water-suppressed scan was acquired from the same VOI.

Spectra were inspected by two investigators (ND and HEK), blinded for the diagnosis. Spectra showing clear artefacts were excluded from further analysis. Quantification of the spectra was performed using LCModel [24–29] (see supplementary methods), in which values of the Cramér-Rao lower bound (CRLB) of metabolite concentrations <20% were considered reliable estimates. If the CRLB of a metabolite exceeded 20% in more than 50% of the cases, that metabolite was excluded from further analysis.

Student's T-tests were used to assess differences between DMD and control groups in age as well as grey matter, white matter and cerebrospinal fluid composition of the VOIs. If these differed significantly, they were used as a covariate in the general linear model that assessed differences in metabolite concentrations. F-tests were used to assess differences in the variance of metabolite concentrations between DMD and control groups. Bonferroni-Holmes corrections for multiple comparisons were used ($p < 0.05$).

RESULTS

Two participants (DMD) were excluded due to corrupted datasets. For the frontal cortex, 3/35 MRS scans (two DMD, one control) failed visual inspection or CRLB criteria. For the hippocampus, two patients did not complete the scan and 4/33 scans (three DMD, one control) failed quality control. For

Table 1
Participant characteristics of the data that was included in the statistical analysis. Metabolite concentrations are shown in mmol/l for the three VOIs with the mean and standard deviation

	MRS frontal cortex		MRS hippocampus		MRS cerebellum	
	DMD	Controls	DMD	Controls	DMD	Controls
Participants (*n*)	16	16	13	15	13	16
Age (years)	12.0 (3.1)	12.9 (2.0)	12.8 (3.1)	12.6 (2.0)	12.9 (3.2)	12.8 (2.1)
Range (years)	8–18	8–16	8–18	8–16	8–18	8–16
Steroid treatment (*n*)	15	–	12	–	12	–
On/off 10 day treatment regimen (*n*)	14	–	11	–	12	–
Brooke scale	1.9 (1.5)	1	2.1 (1.6)	1	2.4 (1.8)	1
Vignos scale	5.9 (3.2)	1	6.3 (3.0)	1	6.2 (3.2)	1
Wheelchair bound (*n*)	9	–	7	–	7	–
Age of loss of ambulation (years)	10.5 (1.3)	–	10.6 (1.4)	–	10.7 (1.3)	–
Regular education (*n*)	9	16	9	15	8	16
N-acetyl-aspartate mmol/L ± SD	$11.3 \pm 1.4^{*}$	10.2 ± 1.2	9.7 ± 1.4	9.4 ± 1.6	13.0 ± 2.4	12.9 ± 2.7
Creatine mmol/L ± SD	8.6 ± 1.0	8.2 ± 1.0	9.8 ± 1.0	9.4 ± 1.6	8.9 ± 1.4	8.9 ± 1.5
Choline mmol/L ± SD	2.0 ± 0.2	2.0 ± 0.2	$2.7 \pm 0.3^{\#}$	2.7 ± 0.5	2.2 ± 0.2	2.2 ± 0.4
Glutamate mmol/L ± SD	$11.4 \pm 2.3^{\#}$	10.2 ± 1.0	10.9 ± 1.9	10.4 ± 1.8	9.6 ± 1.9	10.1 ± 2.5
Glutamate + glutamine mmol/L ± SD	$11.7 \pm 2.5^{\#}$	11.1 ± 1.1	12.5 ± 2.0	12.2 ± 2.2	10.5 ± 2.3	11.2 ± 2.5
Myo-Inositol mmol/L ± SD	4.4 ± 0.7	4.3 ± 0.6	7.1 ± 1.1	7.7 ± 1.8	6.1 ± 1.8	6.7 ± 1.5
Aspartate mmol/L ± SD	5.6 ± 1.5	4.8 ± 1.1	$5.6 \pm 1.5^{\#}$	6.3 ± 2.8	–	–

$^{*}p = 0.02$ before correction for multiple comparisons: not significant after correcting for multiple comparisons. $^{\#}$F test to compare variances: significantly different variances at $p < 0.05$.

the cerebellum, one control did not complete the scan and 4/31 (three DMD, one control) failed quality control. Table 1 summarizes the MRS scans that passed quality control.

There was no significant difference in age between the DMD and control group. Six patients were included who had a distal mutation in the *DMD* gene, which was predicted to affect Dp140 in addition to Dp427 expression.

Representative spectra and metabolite concentrations are shown in Fig. 1, and the means and standard deviations in Table 1. Overall, there were no differences between DMD and control boys in any metabolite concentrations. The variance in glutamate concentration was larger in the frontal cortex compared to healthy controls, while the variance in choline and aspartate in the hippocampus was smaller (Table 1). No correlations with age or grey/white matter fraction were found. Visually, Dp140- patients had similar metabolite concentrations compared to both other boys with DMD and controls (Fig. 1).

DISCUSSION

Our results show preserved biochemical composition of the main metabolites that can be detected using MRS in three localized regions (cerebellum, hippocampus and frontal cortex) of the brain in DMD patients. These areas are known to express dystrophin in healthy controls.

Because dystrophin is associated with $GABA_A$, and glutamate is required both for its synthesis and the product of its breakdown, one hypothesis tested in this work is that the absence of dystrophin would lead to disrupted glutamate metabolism. Indeed, increased glutamate has been previously reported in the temporo-parietal cortex in DMD patients [20]. Interestingly, while the glutamate concentrations in our groups were not significantly different on average, there was a significantly larger variance in our DMD group compared to controls in the frontal cortex. This suggests that glutamate metabolism may be affected on an individual level in DMD. Further research is required to determine whether this is related to the heterogeneity in cognitive performance.

Previous MRS studies in DMD patients have reported increased NAA and conflicting (both increases and decreases) results for choline in different areas of the brain in DMD [18–20]. These studies were conducted at lower field strengths, in smaller cohorts and used less stringent statistics compared to our study. We also find elevated ($p = 0.02$) NAA in the frontal cortex of similar magnitude to the elevated NAA that was reported by Kreis and colleagues in the temporo-parietal cortex [20]. However, the NAA elevation was not statistically significant after correcting for multiple comparisons. We found very similar choline concentrations between patients and controls, which is in contrast to previous findings of both increased [18, 19] and decreased [20]

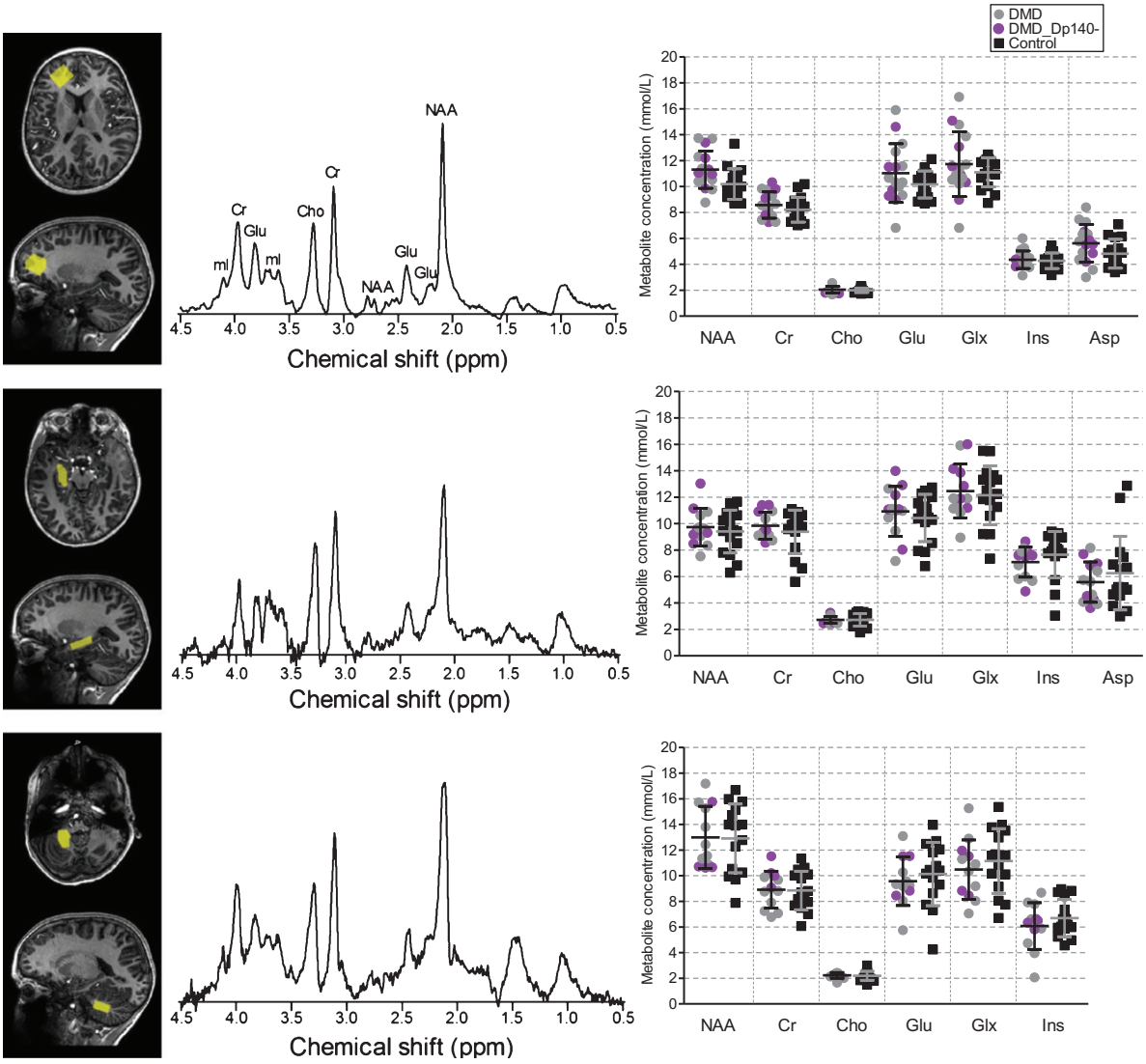

Fig. 1. T1-weighted MRI images are displayed with the VOI indicated by a yellow box. From top to bottom the frontal cortex, hippocampus and cerebellum are shown. Representative spectra are shown in the middle column. Results of the group metabolite concentrations are depicted on the right. Patients are depicted with dots and controls with squares. The patients missing Dp140 in addition to full length dystrophin are highlighted by purple dots.

choline. As choline is largely taken up as a result of food consumption [30], a plausible explanation for the contrasting findings may be differences in diet between the studies.

In *mdx* mice, an animal model commonly used to study DMD, increased choline was also found, as well as decreased GABA and altered amino-acid concentrations [31–33]. Similar to our results, there were no significant differences in any of the other metabolites and no differences were found in the cerebellum. The increased GABA was found in the hippocampus in absence of significant differences in glutamate. Unfortunately, both GABA and the amino-acids were beyond the detection limit of our study, so no comparison can be made.

With respect to the role of Dp140, the groups are too small to establish statistical significance. Within our study, the metabolite concentration values of boys missing Dp140 consistently lie in the same range as for the other boys with DMD which is in line with the earlier study where no effect of Dp140 was found on the metabolite concentrations [20].

To conclude, we have shown preserved biochemical composition, with a statistically significant increased variance in glutamate concentration in boys with DMD compared to healthy controls. These measurements were performed at steady-state and it is possible that metabolism is normal at rest, but changes with challenges that require higher cognitive functioning. To this end, we propose the use of functional MRI or MRS to better assess brain metabolism in response to a challenge. Additionally, the increased variance in glutamate requires further attention as this may be related to the association of Dp427 to $GABA_A$Rs.

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Contributions

The study was set up by CS, HK, JV, JH and EN. Participants were recruited by ND and CS. ND, HK and MH did the data acquisition. ND, MH, SS, AW, CS, MvB, JV, JH, EN and HK were involved in the data discussion. Statistical analyses were aided by EvZ. Data analyses were performed by ND, MH, SS and HK. Writing of the manuscript was performed by ND. Editing of the manuscript was performed by HK, EN, MH, JV and AW. The manuscript was reviewed by all authors.

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The sponsors, Duchenne Parent Project NL and Gratama Stichting, had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

ND reports grants from Duchenne Parent Project and grants from Gratama Stichting during the conduct of the study; MH reports a grant from ZonMW outside of the submitted work; JV, EN and CS report grants from Duchenne Parent Project, ZonMW and AFM, and trial support from BioMarin, GSK, Lilly and Santhera, outside the submitted work. JV reports grants from European Union and consultancy for Biomarin. EN reports consultancies for BioMarin and Summit. HK reports grants from ZonMW, AFM, Duchenne Parent Project, and Gratama Stichting, and consultancy for BioMarin and aTyr Pharma, outside the submitted work. All reimbursements were received by the LUMC. No personal financial benefits were received. All other authors have nothing to declare.

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: http://dx.doi.org/10.3233/JND-160201.

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