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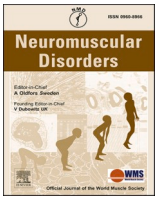

Genotype and corticosteroid treatment are distinctively associated with gray matter characteristics in patients with Duchenne muscular dystrophy

Sam Geuens [a,b,*], Jeroen Van Dessel [c], Hermien E. Kan [d,e], Rosanne Govaarts [d,e], Erik H. Niks [e,f], Nathalie Goemans [a], Jurgen Lemiere [g,h], Nathalie Doorenweerd [d], Liesbeth De Waele [a,b]

[a] University Hospitals Leuven, Child Neurology, Leuven, Belgium
[b] KU Leuven, Department of Development and Regeneration, Leuven, Belgium
[c] Center for Developmental Psychiatry, Department of Neurosciences, UPC-KU Leuven, Belgium
[d] Leiden University Medical Center, C.J. Gorter MRI Center, Department of Radiology, Netherlands
[e] Duchenne Center Netherlands
[f] Leiden University Medical Center, Department of Neurology, Netherlands
[g] University Hospitals Leuven, Pediatric Hemato-Oncology, Belgium
[h] KU Leuven, Department Oncology, Pediatric Oncology, Belgium

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ABSTRACT

This study investigated if structural variation in specific gray matter areas is associated with corticosteroid treatment or genotype, and if cerebral morphological variations are related to neuropsychological and behavioral outcomes. The CAT12 toolbox in SPM was used for MRI segmentations, assessing subcortical structures, cortical thickness, gyrification, and sulci depths for DMD patients (n = 40; 9–18 years) and age-matched controls (n = 40). Comparisons were made between DMD vs. controls, daily vs. intermittent corticosteroid treatment (n = 20 each), and Dp140^{+} vs. Dp140^{-} gene mutations (n = 15 vs. 25). MANCOVA, CAT12 3D statistics and Pearson correlations were conducted. DMD patients showed differences in volumes of distinct subcortical structures, left hemisphere cortical thickness, and gyrification in multiple brain areas compared with healthy controls. The daily treated DMD group exhibited differences in subcortical volumes and different patterns of cortical thickness, sulci depth, and gyrification compared to the intermittent treated DMD group. DMD Dp140^{+} patients displayed altered gyrification and sulci depth compared to DMD Dp140^{-} patients. Finally, we found correlations between neurobehavioral outcomes and brain areas that showed differences in cortical morphology associated with corticosteroid treatment. Both genotype and corticosteroid treatment are associated with variations in subcortical volumes and cortical morphology, albeit in different ways. Corticosteroid treatment appears to have a more profound association with differences in gray matter characteristics of brain regions that are associated with functional outcomes.

1. Introduction

In addition to the severe and progressive muscular loss, patients with Duchenne Muscular Dystrophy (DMD) have an increased risk of developing neurobehavioral problems [1]. This significantly impacts the daily functioning of patients and contributes to a substantial burden on patients and caregivers [2]. The etiology of the neurobehavioral problems has been associated with the absence of dystrophin expression in the brain, but till now effective treatments for these problems remain elusive [3].

Dystrophin, a protein encoded by the x-linked *dystrophin* gene, is expressed in various tissues throughout the body [4]. DMD patients have a mutation in this gene resulting in dystrophin protein absence. The lack of dystrophin in muscles results in replacement of muscle tissue by fat and fibrosis and functional decline as the hallmark of the disease [5]. In addition to muscle, isoforms of dystrophin are expressed in the brain during different stages of development [6]. The functions of these isoforms in the brain remain unclear and gaining an understanding is beneficial to improve management of neurobehavioral problems in patients with DMD [2].

Structural brain MRI studies have consistently revealed smaller total gray matter volumes and less structured white matter networks in

* Corresponding author at: Child Neurology, University Hospitals Leuven, Herestraat 49, 3000 Leuven, Belgium.
E-mail address: sam.geuens@uzleuven.be (S. Geuens).

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patients with DMD compared to healthy controls [3,7–9]. These findings also showed that patients with mutations located more distally in the *dystrophin* gene, lacking brain isoform Dp140, exhibit more pronounced brain alterations [7,8]. In addition, prior research indicated that corticosteroids, a standard treatment initiated in DMD patients from a young age to slow down disease progression, may represent a confounding factor in this context [10]. Corticosteroids can cross the blood-brain barrier, and it has been shown that this can lead to structural alterations [10–14]. In general, growing evidence suggest that brain research in DMD is subject to complex variations within the DMD population linked to genetic or environmental factors [7,9,10]. The first objective of the current study is to investigate which parts of the gray matter are affected in DMD and whether structural patterns are associated with corticosteroids regimen, or with genotype. Secondly, our objective is to explore the clinical relevance of cerebral morphological variations within the DMD population by examining their relationship with neuropsychological and behavioral outcomes.

2. Material and methods

2.1. Participants

As reported in Geuens et al. [10]., we enrolled 40 individuals with DMD from two medical centers, UZ Leuven (UZL) in Belgium, and Leiden University Medical Center (LUMC) in the Netherlands, and a healthy control group comprising 40 age-matched participants (20 from UZL, 20 from LUMC) in accordance with following inclusion criteria:

1) age between 9 and 20 years;
2) native Dutch speaking;
3) genetically confirmed diagnosis of DMD with specification of gene mutation;
4) treatment with corticosteroids following the clinical practice guidelines.

Patients with DMD were either treated daily with deflazacort (0.9 mg kg^{-1}/day with a maximum of 36 mg per day; $n = 20$ from UZL) or intermittently (10 days on/10 days off) with prednisone (0.75 mg kg^{-1}/day, with a maximum of 30 mg per day; $n = 20$ from LUMC) [15].

Ethical approval was obtained from both centers (UZL: s62667 and LUMC: P09.121). Prior to the scanning procedures, participants and their parents provided written informed consent. Data management and processing adhered to the regulations of the Helsinki Convention and the General Data Protection Regulation ensuring ethical standards and participant confidentiality were upheld throughout the study.

2.2. Demographic and clinical information

The age of participants at the time of scanning was recorded for the entire study cohort. For individuals with DMD, additional clinical information was extracted from the patient records. This information included ambulation status, age at initiation of corticosteroid treatment, and details on genetic diagnosis. Participants were classified as wheelchair-bound if they had lost the ability to walk independently for >10 m at the time of the scanning session. Data on the initiation of corticosteroid treatment were successfully obtained for 20 patients in the daily treated group, and for 12 patients in the intermittently treated group. For eight patients in this group, treatment history data were incomplete due to records dating back to a period when record-keeping practices were suboptimal. This information formed the basis for calculation of the duration of corticosteroid use up to the scan date. The specific details about genetic diagnosis were used to divide the DMD patients in a $Dp140^{+}$ and a $Dp140^{-}$ group, using the universal DMD mutation database, a French knowledge base derived from functional studies predicting the effect of several mutations (http://www. umd.be/DMD/4DACTION/W_ISO/L) [16]. Patients in the $Dp140^{+}$ group have a mutation upstream of intron 44, which suggests the ability to produce the brain dystrophin isoform Dp140 [1]. Patients in the $Dp140^{-}$ group have a mutation downstream of intron 44 and are assumed to lack the brain dystrophin isoform Dp140 [1].

2.3. MRI protocol

MRI scanning was performed on a 3T Philips Achieva scanner (Philips Healthcare Best, the Netherlands) with a 32-channel head coil (UZL) or an 8-channel head coil (LUMC). Structural scans were acquired using a standard T1-weighted pulse sequence with fixed scan parameters: TE = 4.6 ms, TR = 9.7 ms, flip angle = 8°, field of view = 256 × 242 mm^2 and 1 mm^3 voxel size. Scans were assessed by an independent radiologist for gross structural abnormalities. All scans were conducted by trained researchers. Prior to being positioned in the MRI scanner, participants were familiarized with the scanner and scan room, and they received a detailed explanation suitable for their age and comprehension level.

2.4. Neuropsychological and behavioral testing

Full Scale Intelligence Quotient (FSIQ) for DMD patients scanned at UZL was obtained using the Wechsler intelligence scale for Children, fifth edition, Dutch (WISC-V-NL) [17]. FSIQ for DMD patients at LUMC was estimated based on the Peabody Picture Vocabulary test (PVVT-III-NL) [7]. Behavioral functioning was quantified in both groups by means of the total scale scores, internalizing scale scores and externalizing scale scores of the Child Behavior Checklist (CBCL) [18].

Neuropsychological testing was done by a trained researcher (SG at UZL and ND at LUMC) on the day of the scan, prior to the MRI procedure. At the same time, the CBCL was completed by one of the parents.

2.5. Structural analysis

Gray matter- and surface-based analyses were performed using the Computational Anatomy Toolbox (CAT12) (http://dbm.neuro.uni-jena.de/cat/), an automated approach to measure subcortical volumes, and to estimate cortical thickness and reconstruct the hemispheric central surface using a projection-based thickness method [19]. The processing pipeline includes brain segmentation into gray matter, white matter and cerebrospinal fluid, affine registration to MNI template space, and subsequent nonlinear deformation. For measurement of cortical thickness, segmentation was used to estimate white matter distances followed by projection of local maxima to other gray matter voxels using neighborhood relationships. Two additional surfaces measures, gyrification and sulcal depth, were extracted as well. Based on the absolute mean curvature, gyrification index was calculated as a ratio of external brain surface over outer surface excluding the sulci [20]. The sulcus depth with square root function transformation, making the data more normally distributed, was defined as the Euclidean distance between the central surface and the convex hull [21].

The segmented gray matter images and the surface images were smoothed using a Gaussian kernel with a full-width-half-maximum (FWHM) of 6 mm for gray matter, 15 mm for cortical thickness and 20 mm for gyrification and sulcal depth, respectively [19].

A data quality check was performed after segmenting the raw T1-images with image quality ratio (IQR), visual inspection of the gray matter segmentations by SG and JVD, and examination of the homogeneity of the sample. IQR of all files turned out to be average or above average (average IQR= 80.77 %, SD=4 %). Visual inspection of all gray matter segmentations did not identify any abnormalities during normalization (Fig. 1). The sample homogeneity check revealed three outliers. Visual inspection of these outliers revealed that this difference was not due to artefacts or issues with segmentation, but to natural variation. All scans passed the data quality check and were included for further analyses.

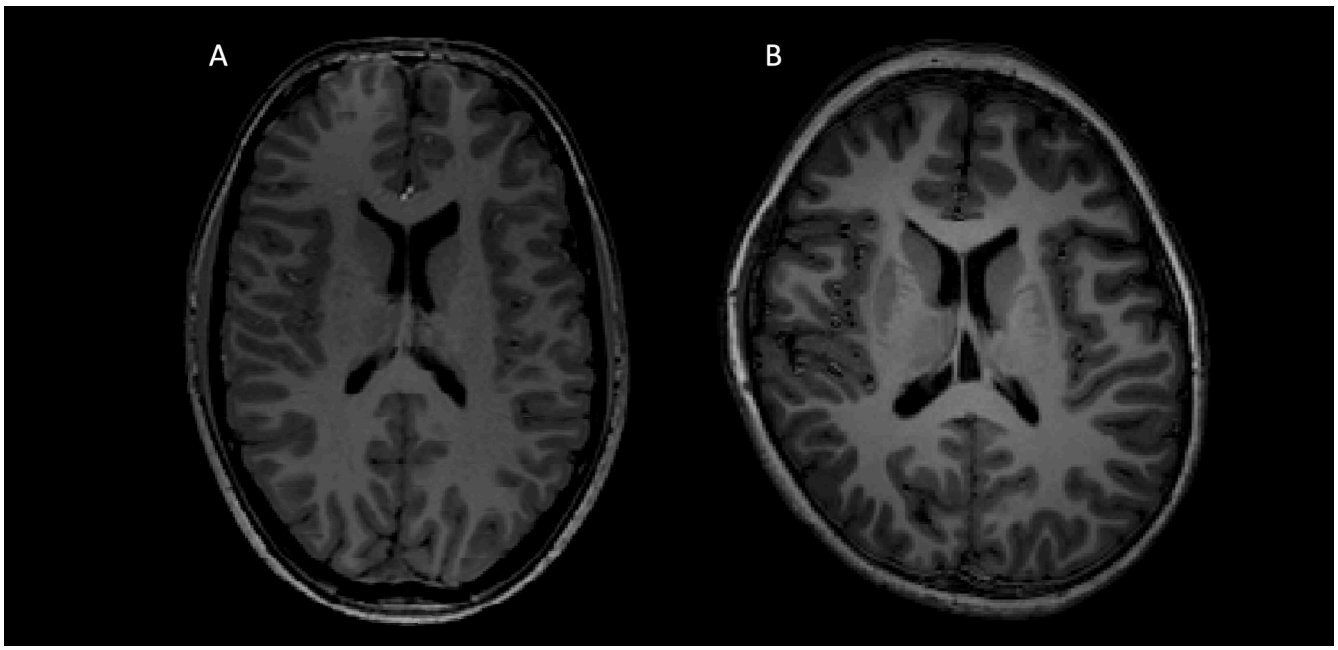

Fig. 1. Representative T1-weighted images.
An age-matched healthy control (A) and a patient with DMD (B).

2.6. Statistical analysis

For the first research objective, all analyses were performed separately for three conditions: 1) comparison of all DMD patients with the control group (condition = case control), 2) comparison of the daily treated DMD patients with the intermittently treated DMD patients (condition = corticosteroid treatment), and 3) comparison of the $Dp140^+$ patients with the $Dp140^-$ DMD patients (condition = genotype).

Independent sample *t*-tests were used to assess differences in age for the three conditions. Next, three distinct analyses were done using a one model multivariate analysis of covariance (MANCOVA) with subcortical volumes as dependent variables, total intracranial volume (TIV) as covariate and the three conditions as independent variables. Statistical analyses were performed in SPSS (version 28, IBM, New York, USA) at a significance level of 0.05.

In addition, for each condition, analyses for cortical thickness, gyrification and sulci depth were performed in CAT12 using two-sample *t*-tests on the left and right hemispheres separately. Atlas-based multiple comparisons were conducted with the family-wise error (FWE) method and a cluster threshold of $p = 0.001$ (FDR-corrected). An additional correction was used to account for the three conditions based on a Bonferroni adjustment of a significance level of 0.05, resulting in a significance level for region of interest analyses on 0.017 [22].

To answer the second research question, Pearson correlation coefficients (r) were calculated between neuropsychological and behavioral variables and the volumes of the subcortical structures and the cortical morphological parameters of those brain areas that turned out to be significantly different within the different DMD conditions. Given the exploratory nature of this study and the large number of brain areas analyzed, we did not apply multiple comparison corrections. Correlations were considered significant at $p < 0.05$. Correlation strength was interpreted as strong (> 0.7), moderate (0.4 to 0.7), or low (< 0.4) [23].

3. Results

3.1. Participants

There were no differences in age between the groups of the three specified conditions (Table 1).

Table 1
Clinical and demographical characteristics of participants, grouped by condition.

Control		DMD			
		Corticosteroid treatment		Genotype	
		Daily[b]	Intermittent[c]	$Dp140^+$	$Dp140^-$
No. of participants	40	20	20	15	25
Age, years[a]	13.0 ± 2.4	13.2 ± 3.2	13.0 ± 3.1	13.2 ± 3.7	12.9 ± 2.8
Age range, years	9.0 – 18.0	9.5 - 18.9	9.1 - 18.3	9.1 – 18.6	9.8 – 18.9
Scan site, UZL/LUMC	20/20	20/0	0/20	6/9	14/11
Wheelchair bound	NA	6	13	7	12
Duration of corticosteroid treatment, months[a]	NA	81 ± 30	79 ± 35	86.9 ± 39.7	76.9 ± 26.9
$Dp140^+/Dp140^-$	NA	6/14	9/11	15/0	0/25

DMD = Duchenne muscular dystrophy.
[b] Daily = treated with daily deflazacort.
[c] Intermittent = treated with 10 days on/10 days of prednisone; NA = not applicable; $Dp140^+$ = DMD patients with a gene mutation allowing production of brain dystrophin Dp140; $Dp140^-$ = patients with a gene mutation prohibiting production of brain dystrophin Dp140.
[a] Data are presented as mean with standard deviation.

3.2. Subcortical volumes

The MANCOVA showed significant ($F(28,50) = 2.023$, $p < 0.05$, Wilks' $\Lambda = 0.463$, partial $\eta 2 = 0.531$) group differences between the control group and the DMD group for volumes of the 3rd ventricle, right amygdala, brain stem, exterior of both cerebellar hemispheres and bilateral lateral ventricles (Table 2).

Within the DMD group, we found a significant effect for corticosteroid regime ($F(28,8) = 3.858$, $p < 0.05$, Wilks' $\Lambda = 0.069$, partial $\eta 2 = 0.931$) but not for genotype ($F(28,8) = 0.340$, $p = 0.99$, Wilks' $\Lambda = 0.457$, partial $\eta 2 = 0.543$). Patients treated daily showed significantly higher volumes of the 4th ventricle, the left exterior of the cerebellum,

Table 2
Significant subcortical volumetric differences between the control group and the DMD group.

Characteristic	Control (n = 40)	DMD (n = 40)	F-value
3rd ventricle, cm^3	0.20 ± 0.07	0.15 ± 0.07	6.04**
Right amygdala, cm^3	0.98 ± 0.07	0.91 ± 0.15	4.26*
Brain stem, cm^3	1.45 ± 0.30	1.68 ± 0.49	12.95***
Exterior of the right cerebellum, cm^3	49.07 ± 3.12	45.98 ± 4.16	9.87**
Exterior of the left cerebellum, cm^3	48.85 ± 3.13	46.02 ± 4.38	7.15**
Right lateral ventricle, cm^3	1.10 ± 0.27	0.89 ± 0.22	13.25***
Left lateral ventricle, cm^3	1.23 ± 0.32	0.98 ± 0.24	14.11***

Note: data are presented as mean with standard deviation.
DMD = Duchenne muscular dystrophy;.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

bilateral putamen and bilateral thalami and a lower volume of the brain stem compared to patients who are treated intermittently (Table 3).

3.3. Cortical morphology

3.3.1. Cortical thickness

We found significant decreases of cortical thickness in the DMD group compared with the control group in the left frontal cortex, left inferior and middle temporal cortex, and the left superior parietal cortex. In the right hemisphere, only the medial orbitofrontal cortex showed lower values of cortical thickness in the DMD group compared with the control group (Fig. 2A).

Within the DMD group, significant increases in cortical thickness were observed between DMD patients undergoing daily corticosteroid treatment and those treated intermittently. Specifically, significant differences were noted in the left frontal and temporal regions around the lateral sulcus, as well as in the left occipital regions. In the right hemisphere, differences were observed in the frontal pole, lateral orbitofrontal cortex, cingulate cortex, pre- and postcentral cortex, pars triangularis, and pars opercularis, along with the inferior, middle, and superior temporal cortexes, supramarginal cortex, and inferior parietal cortex. Additionally, differences were found bilaterally in the lateral occipital, cuneus, lingual, and fusiform cortex (Fig. 2B).

We found no significant effect of genotype on cortical thickness within the DMD group.

3.3.2. Gyrification

We observed significantly higher gyrification indexes in different areas in the left middle temporal gyrus and the right gyrus rectus in DMD patients compared with the control group (Fig 3A). We found significantly lower gyrification indexes for the daily treated DMD patients compared to the intermittently treated DMD patients in the bilateral temporal poles, supramarginal gyri and insular gyri, as displayed in Fig. 3B. Significantly decreased (blue) gyrification indexes were found in the DMD $Dp140^+$ patients compared with DMD $Dp140^-$ patients in the inferior parietal lobule (Fig. 3C).

Table 3
Significant subcortical volumetric differences between daily and intermittently treated DMD patients.

Characteristic	Daily (n = 20)	Intermittent (n = 20)	F-value
4th ventricle, cm^3	0.23 ± 0.10	0.17 ± 0.05	5.97*
Brain stem, cm^3	1.46 ± 0.38	1.90 ± 0.50	8.79**
Exterior of the left cerebellum, cm^3	46.79 ± 4.12	45.24 ± 4.61	4.29*
Right Putamen, cm^3	4.40 ± 0.28	4.23 ± 0.42	7.58**
Left Putamen, cm^3	4.55 ± 0.33	4.40 ± 0.50	8.91**
Right Thalamus, cm^3	6.10 ± 0.48	4.88 ± 0.61	54.56***
Left Thalamus, cm^3	5.82 ± 0.41	4.80 ± 0.54	49.19***

Note: data are presented as mean with standard deviation.
DMD = Duchenne muscular dystrophy; Daily = treated with daily deflazacort; Intermittent = treated with 10 days on/10 days off prednisone.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

3.3.3. Sulci depth

No significant differences in sulci depth were observed between DMD patients and the control group. However, within the DMD group, decreased values of sulci depth were noted for individuals treated daily compared to those treated intermittently with corticosteroids for the sulci of the bilateral frontal and temporal poles, bilateral cingulate sulci, bilateral intraparietal sulci, as well as the left hemispheric superior parietal sulcus and superior frontal sulcus, as illustrated in Fig. 4A.

Fig. 4B displays significantly increased (red-yellow) and decreased (blue) sulci depth in the right hemispheric pars triangularis, superior parietal sulcus, the lateral occipital sulcus, the precuneus sulcus and the insular sulcus in the $Dp140^+$ group compared with the $Dp140^-$ group.

3.4. Neuropsychological and behavioral outcomes

We did not identify any moderate to strong correlations between the subcortical volumes that exhibited significant differences in the corticosteroid treatment condition, and neuropsychological and behavioral outcomes. However, we found several significant moderate correlations between FSIQ and the CBCL scale scores and the cortical morphological measurements of brain areas that had significant differences found in the corticosteroid treatment condition and the genotype condition (Table 4).

4. Discussion

Our results demonstrate the complexity of disentangling contributing factors to altered brain morphology in DMD. Distinct subparts of the cerebral gray matter are differently affected in DMD and both genetic and treatment-related factors appear to be associated, each in a specific manner, to substantial variations in gray matter patterns. Together, these patterns may provide distinguishing biomarkers, but determining the exact composition of the signature patterns requires international collaboration to include a wide variety of individuals. Gaining a more profound understanding of these patterns is essential, given the apparent association with neuropsychological and behavioral functioning.

4.1. DMD versus control

Previous research primarily investigated differences in general gray matter volume between patients with DMD and healthy participants through whole-brain analyses [7,10,24,25]. Our results are in line with this, as we found significantly decreased gray matter volumes in specific subcortical structures in DMD patients compared with healthy controls, including the exterior of both cerebellar hemispheres. Cerebellar involvement in DMD has been previously demonstrated and posited as a potential explanation for specific neurocognitive deficits, as the cerebellum harbors a significant concentration of Purkinje cells in which dystrophin is expressed in substantial amounts in healthy individuals [26,27].

Our findings of reduced volumes in the right amygdala are the first to link amygdala involvement to DMD. Typically, reduced amygdala volumes are associated with increased exposure to stress, disrupted emotional regulation, and a propensity for anxious and socially anxious behavior [28]. Given the frequent reports of anxiety issues in DMD, further exploration of the role of the amygdala in the context of DMD is

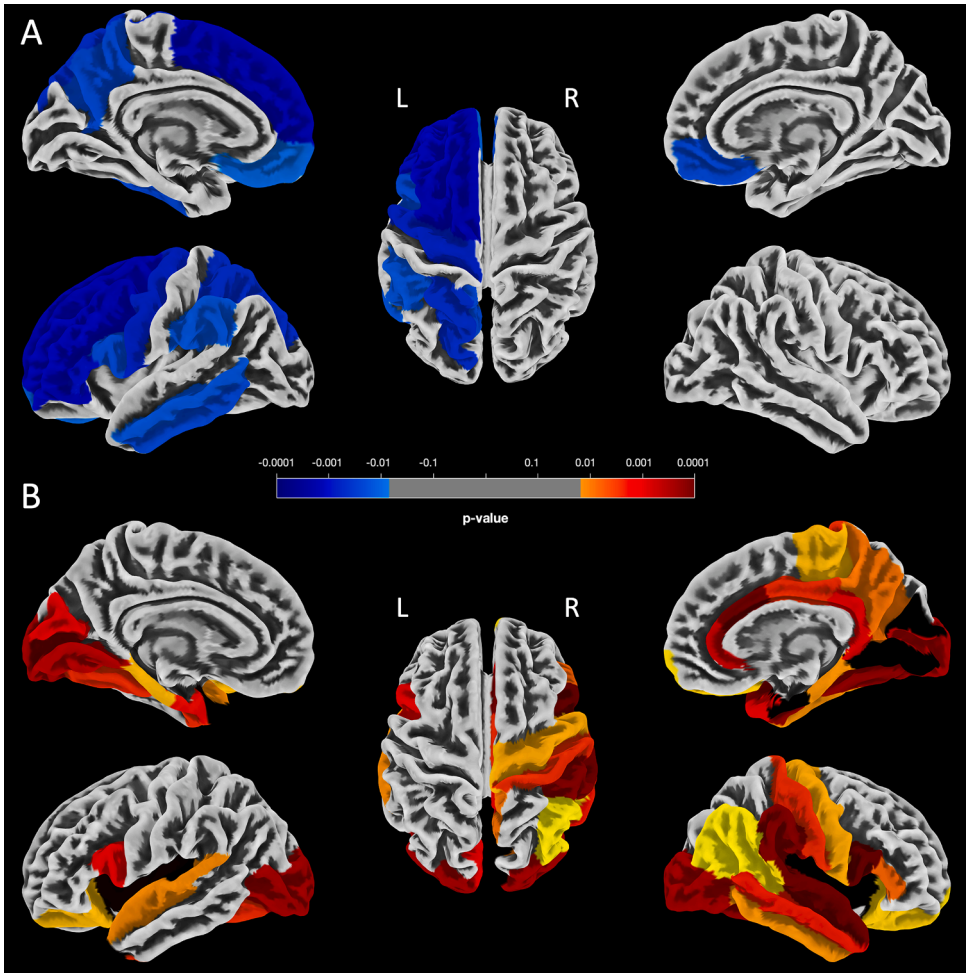

Fig. 2. Cortical thickness.
(A) Brain areas with decreased cortical thickness (blue) in DMD patients ($n = 40$) compared with healthy controls ($n = 40$) and (B) brain areas with increased cortical thickness (red - yellow) in daily treated DMD patients ($n = 20$) compared with intermittently treated DMD patients ($n = 20$).

warranted [29].

In addition to gray matter volume differences, our results demonstrated decreased cortical thickness and, to a lesser degree, increased cortical gyrification in distinct brain areas in individuals with DMD compared to healthy controls. Notably, the cortical thinning in DMD patients was predominantly restricted to the left hemisphere. Deviant cortical thickness is associated with lower intellectual outcomes, neurodevelopmental disorders, and psychiatric conditions such as anxiety disorders, schizophrenia, and autism spectrum disorder [30–33]. However, these findings are linked to cortical thinning in both hemispheres, leaving the profound asymmetry in our sample unexplained. Given that most participants were right-handed ($n = 74$), we may assume that most left hemispheres in this sample are involved in speech, language, and processing of words, letters, and numbers [34]. These are functions associated with difficulties in individuals with DMD, but further investigation is needed to ascertain if and how these are related to cortical morphological deviations.

4.2. Corticosteroid treatment

Within the DMD population, patients treated with a daily corticosteroid regimen exhibited larger volumes in certain subcortical structures, including the thalamus and putamen bilaterally. While these volumes were not directly associated with neurobehavioral outcomes in our sample, it is noteworthy that both increased and decreased thalamus volumes have been linked to behavioral abnormalities in other studies [35]. Accordingly, enlarged putamen volumes are consistently associated with obsessive-compulsive disorders and increased levels of anxiety [36]. We found no evidence that brain stem or ventricle sizes were linked to functional outcomes, in contrast to findings in other pediatric populations [37]. The mechanism by which corticosteroid treatment contributes to different subcortical volumes in DMD patients remains elusive, as most research indicates that overexposure to corticosteroids affects hippocampal areas and the limbic system, which was not observed in our cohort [38–40].

The finding of increased cortical gray matter thickness in several brain areas among daily treated patients may appear contradictory, given our previous results in this same cohort where we showed a general decrease in total gray matter volumes among DMD patients receiving daily corticosteroid treatment compared to those treated intermittently [7,10]. However, cortical thickness is only one dimension of volume and measured as the distance from the pia to the gray and white matter border [19]. During child development, there is a natural process of synaptic pruning and myelin proliferation, which leads to white matter expansion that replaces gray matter and results in age-related reductions in cortical thickness [41–44]. Consequently, the observed differences in cortical thickness may indicate a lesser degree of white matter maturation in daily treated patients. This aligns with our previously proposed hypothesis that a daily corticosteroid regimen interferes more profoundly with natural cortisol-induced brain maturation processes compared to a corticosteroid regimen that allows the periodic resumption of natural production [10].

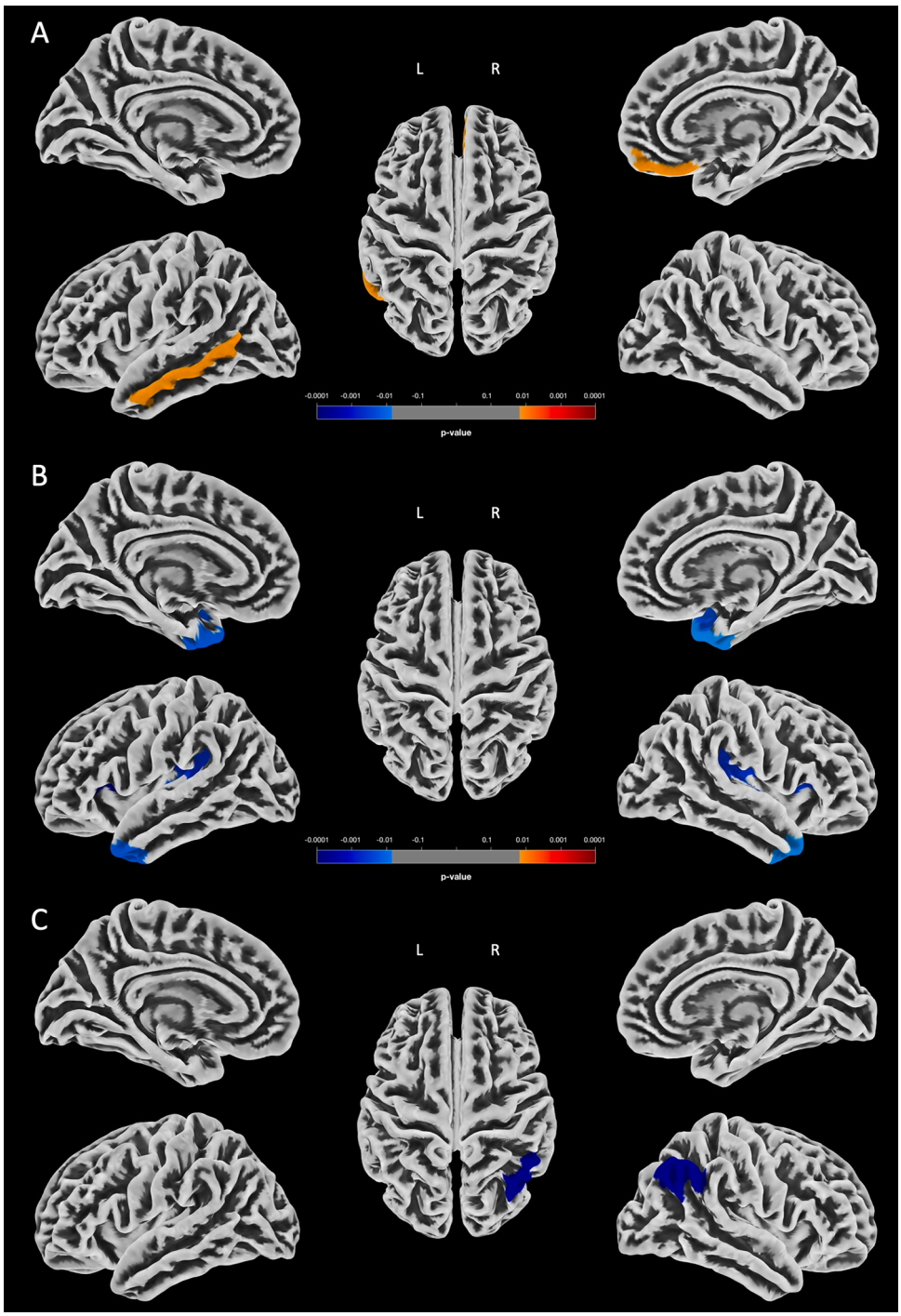

Fig. 3. Gyrification.
(A) Brain areas with increased gyrification indexes (red - yellow) in DMD patients ($n = 40$) compared with healthy controls ($n = 40$), (B) brain areas with decreased gyrification indexes (blue) in daily treated DMD patients ($n = 20$) compared with intermittently treated DMD patients ($n = 20$) and (C) brain areas with decreased (blue) gyrification indexes in $Dp140^{+}$ DMD patients ($n = 15$) compared with $Dp140^{-}$ DMD patients ($n = 25$).

The predominantly observed increase in cortical thickness in the right hemisphere may offer a partial explanation for the previously discussed finding of an overall decreased cortical thickness in the left hemisphere among the entire DMD patient group. Specifically, when comparing only the intermittently treated DMD patients with the control group, cortical thickness was similarly reduced in both hemispheres. This observation suggests that the increased cortical thickness observed in the right hemisphere of the daily treated group influences group averages, resulting in a lack of statistical significance. Asymmetries in cortical thickness have been documented in prior research [45]. In our study, we primarily observed these asymmetries in the comparison between different corticosteroid regimens, suggesting a potential effect induced by corticosteroid treatment. Mineralocorticoid and glucocorticoid receptors are ubiquitously expressed throughout the brain. The overexposure and overactivation of these receptors, particularly the glucocorticoid receptors, which are the preferred targets of both prednisone and deflazacort, have been associated with detrimental effects on brain structure and function [46]. It is expected that such effects would

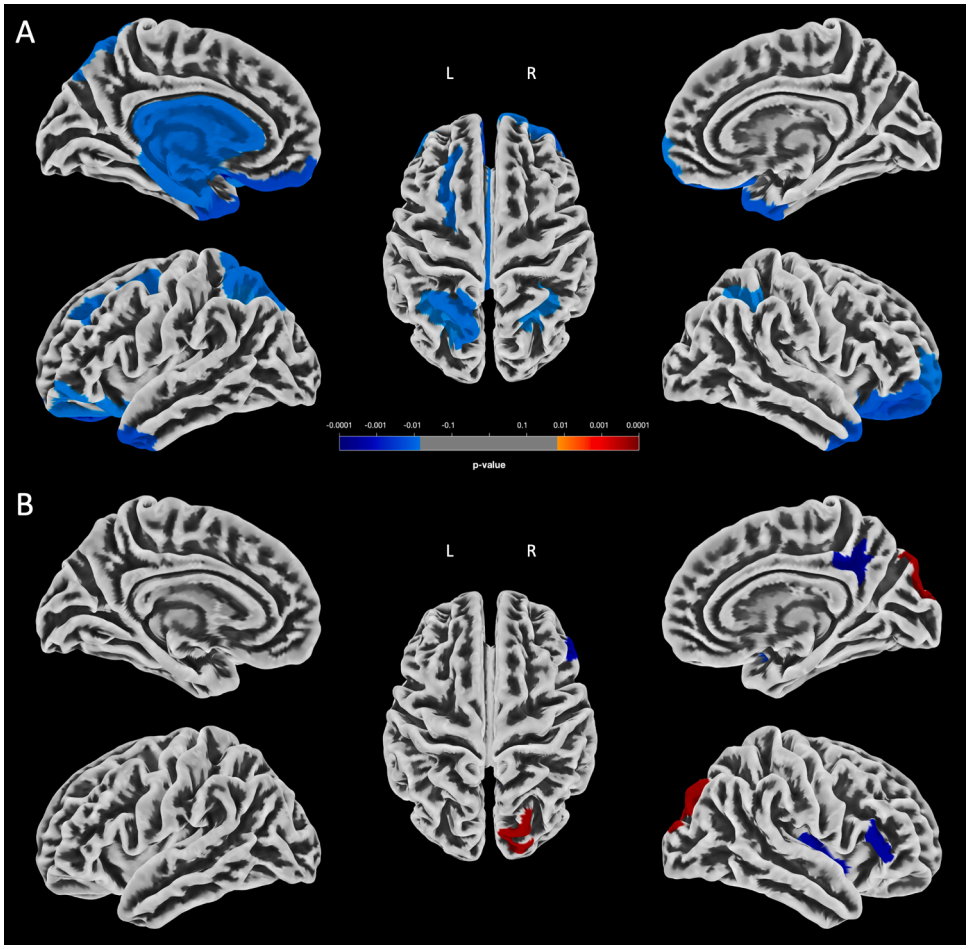

Fig. 4. Sulci Depth.
(A) Brain areas with decreased sulci depth (blue) in daily treated DMD patients ($n = 20$) compared with intermittently treated DMD patients ($n = 20$) and (B) displays brain areas with increased (red-yellow) and decreased (blue) sulci in Dp140^{+} DMD patients ($n = 15$) compared with Dp140^{-} DMD patients ($n = 25$).

manifest globally in the brain, leaving the observed asymmetry in our findings unexplained.

4.3. Genotype

In our analysis, the absence of the brain dystrophin isoform Dp140 resulted in lower gyrification and both increased and reduced sulci depths depending on the location, but not on subcortical volumes or cortical thickness. In contrast, the absence of the full-length brain dystrophin Dp427, which is lacking in all DMD patients, exhibited a significant impact on subcortical volumes and cortical thickness, and to a lesser extent on gyrification, but not on sulci depth [4]. These findings suggest distinct effects of both brain dystrophin isoforms Dp427 and Dp140 on various aspects of the gray matter. This is supported by general findings that the different brain dystrophin isoforms play different roles throughout brain development, making it plausible that the absence of multiple isoforms results in different effects on distinctive aspects of the brain [3].

Our findings indicate that both genotype and corticosteroid regimen, contribute to variations in sulci depth and, to a lesser extent, gyrification within the DMD population. These metrics, which reflect cortical folding and complexity, serve as sensitive indicators of cortical development [21,47]. Cortical folding initiates during fetal development around 20 weeks postmenstrual age and continues in different waves. By the age equivalent to full-term birth, the majority of sulci and gyri are formed, establishing the overall cortical shape, which stays consistent between neonates and adults, with the same folding variability [47]. However, local cortical morphological changes occur throughout a lifetime, influenced by both endogenous and exogenous factors [48,49]. For example, sulci depths, measured as the distance between the cortical surface and its cerebral hull, undergo changes with aging, associated with gray matter atrophy and cognitive decline in elderly people [50]. On the other hand, gyrification indexes appear to remain relatively stable throughout postnatal development [51]. Since cortical folding predominantly occurs during preterm development, the contrast between Dp140^{+} and Dp140^{-} DMD patients in gyrification and sulci depth is intriguing, given that the brain dystrophin isoform Dp140 is primarily expressed during fetal development [6].

4.4. Neuropsychological and behavioral functioning

The mechanisms underlying cortical folding and complexity, as well as their association with neurodevelopment, remain largely unknown [47]. Both increased and decreased sulci depth and gyrification indices have been linked to better or worse cognitive and neurodevelopmental outcomes, depending on the anatomical brain region [47,51,52]. This diversity is reflected in our findings, showing both positive and negative correlations between regional cortical morphological outcomes and neuropsychological and behavioral outcomes. Most of these correlations were found in brain regions that were significantly impacted by corticosteroid treatment.

Future investigations should incorporate longitudinal studies of children, adolescents, and young adults with DMD to trace the trajectory of cortical morphology patterns and neurobehavioral functioning (e.g.

Table 4
Correlations coefficients between significant cortical morphological parameters and neuropsychological and behavioral outcomes.

	FSIQ	CBCL total	CBCL inter.	CBCL exter.	Condition
Cortical thickness					
Right Transverse Temporal	−0.25	0.52**	0.41*	0.44**	CS
Left Insula	−0.42**	0.07	−0.04	0.07	CS
Right Insula	−0.34*	0.46**	0.46**	0.32	CS
Gyrification					
Left Gyrus and Sulcus Cinguli Middle Posterior	−0.11	0.34*	0.14	0.40*	CS
Right Gyrus Temporalis Superior Gracilis and Sulcus Temporalis Transversus	0.25	−0.51**	−0.49**	−0.32	CS
Right Gyrus Temporalis Superior Planum Polare	0.30	−0.44**	−0.35*	−0.34*	CS
Right Pole Temporal	0.48**	−0.38*	−0.35*	−0.30	CS
Left Sulcus Circularis Insulae Inferior	−0.44**	0.11	0.26	0.02	CS
Right Sulcus Circularis Insulae Inferior	−0.05	0.48**	0.49**	0.31	CS
Sulci depth					
Right Gyrus Temporalis Superior Gracilis and Sulcus Temporalis Transversus	0.49**	.498**	0.45**	0.49**	CS
Right Sulcus Occipitalis Middle and Sulcus Lunatus	−0.42*	−0.51**	−0.33	−0.42*	Dp

FSIQ = Full Scale Intelligence Quotient; CBCL = Child Behavior Checklist; total = total scale score; inter. = internalizing scale score; exter. = externalizing scale score; condition = condition in which significant differences were found among DMD patients; CS = corticosteroid treatment; Dp = genotype.
* $p < 0.05$;.
** $p < 0.01$.

intelligence, working memory, social cognition and social responsiveness and anxiety) and to assess the impact of genotype and corticosteroid treatment over time.

4.5. Limitations

Several limitations should be considered when interpreting the findings of our study. Firstly, we used a categorization strategy where DMD patients with a mutation proximal to intron 44 were assumed to produce the brain dystrophin Dp140, while those with a mutation distal to intron 44 were considered unable to produce Dp140, aligning with recommendations from prior research [8,9,16]. It is important to note that this approach is suboptimal, as the production of brain dystrophin Dp140 is unpredictable in DMD patients with mutations between exon 45 and exon 51 ($n = 11$ in our sample). Furthermore, our study could not explore the impact of the smallest brain dystrophin isoform, Dp71, hindered by mutations downstream from exon 63, due to limited participant representation with such mutations. Large sample studies, with sufficient representation of different mutations are needed to shed more light on the role of dystrophin deficiency in the brain. Finally, patients across a broad age range (9 to 18 years) were included. The considerable variation in individual developmental rates within the general population poses a challenge for cross-sectional comparisons, particularly in relatively small cohorts [53]. Although we matched the groups based on age and gender, differences related to socio-economic status or intellectual abilities were not controlled for.

5. Conclusion

In summary, our findings indicate alterations in various aspects of the gray matter in individuals with DMD. Moreover, within the DMD population, both genotype and corticosteroid treatment were associated with variations in subcortical volumes and cortical morphology, albeit in different ways. Corticosteroid treatment appears to have a more profound association with differences in gray matter characteristics of brain regions associated with functional outcomes.

Data availability

The data supporting the findings of this study are available within the article and/or available upon request to the corresponding author.

CRediT authorship contribution statement

Sam Geuens: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Jeroen Van Dessel:** Writing – review & editing, Supervision, Methodology, Formal analysis. **Hermien E. Kan:** Writing – review & editing, Supervision. **Rosanne Govaarts:** Writing – review & editing, Supervision, Methodology. **Erik H. Niks:** Writing – review & editing, Supervision. **Nathalie Goemans:** Writing – review & editing, Supervision. **Jurgen Lemiere:** Writing – review & editing, Supervision, Methodology. **Nathalie Doorenweerd:** Writing – review & editing, Supervision, Methodology, Data curation. **Liesbeth De Waele:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of competing interest

No conflict of interest was reported.

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