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Prefrontal cortical thickness in motor neuron disease

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ABSTRACT

Objective: To examine whether the distribution of prefrontal cortical thickness in patients with motor neuron disease is normal or bimodal and how it compares to the normal population.
Methods: 158 patients with motor neuron disease (MND) and 86 healthy controls (HC) were enrolled in a prospective, two-center study with a common structural MRI protocol. Cortical thickness measures were extracted for the prefrontal cortex, premotor cortex, motor cortex, and occipital cortex using FreeSurfer, adjusted for age and sex, and tested for normality of distribution.
Results: Cortical thickness measures of the bilateral prefrontal, premotor, motor, and occipital cortex were normally distributed in patients and healthy controls. MND-related cortical thinning was observed in the right motor cortex ($p = 0.002$), reflected in a significantly higher proportion of MND cases being worse than −1 standard deviation of the healthy control mean: 29.1% in the right motor cortex ($p = 0.002$). Cortical thinning of the left motor cortex was a function of clinical phenotype and physical disability. Left prefrontal cortical thickness was reduced in patients with additional cognitive and/or behavioural deficits compared to MND patients without cognitive deficits. Prefrontal, premotor, motor, and occipital cortical thickness was related to patients' general cognitive abilities.
Conclusion: The study shows that prefrontal cortical thickness in MND is normally distributed but shifted towards thinner cortex in MND patients with cognitive and/or behavioural impairment. The distribution of thickness values did not indicate the assumption of a bimodal distribution although patients with comorbid cognitive deficits are more likely to suffer from prefrontal cortical thinning.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is characterised by the progressive loss of motor neurons. The heterogeneity in clinical phenotypes is large, manifesting in sporadic or familial forms; varying degrees of upper and lower motor neuron involvement; site of onset; and disease progression (Swinnen and Robberecht, 2014). Up to 50% of the patients show behavioural or cognitive impairment (Phukan et al., 2012), with about 15% fulfilling the criteria for comorbid frontotemporal dementia (FTD) (Montuschi et al., 2015; Phukan et al., 2012; Ringholz et al., 2005).

Although there is compelling evidence for the co-occurrence of ALS and FTD (DeJesus-Hernandez et al., 2011; Neumann et al., 2006; Renton et al., 2011), it remains unclear if patients with ALS generally are at risk of FTD or if only a subgroup. Previous studies have taken advantage of magnetic resonance imaging techniques to compare grey matter volume (Lillo et al., 2012a; Mioshi et al., 2013) and cortical thickness (Schuster et al., 2014) across the spectrum of ALS and FTD. Although these studies report an overlap in atrophy patterns, group averages do not address how individual data points are distributed. FTD comprises a variety of different phenotypes, typically behavioural variant FTD (bvFTD), semantic variant primary progressive aphasia (sv-PPA) and non-fluent variant PPA. Although all variants can occur with ALS, it is the bvFTD phenotype that is most frequently observed in ALS-FTD (Lillo et al., 2012b). One hallmark feature of bvFTD is involvement of the frontal (and temporal) lobes, making prefrontal thinning a possible indicator for being at risk of FTD. This leads to the assumption that if only a sub-group of patients with ALS were at risk of FTD, one

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prediction might be a bimodal distribution with respect to cortical thickness in prefrontal regions — i.e. comprising a population with thinning of the prefrontal cortex indicating risk of FTD and a population with an identical distribution to the healthy population. On the other hand, if the distribution were unimodal, the question arises as to whether it would shift toward thinner cortex in ALS suggesting a more general vulnerability to FTD. This study addressed these scenarios by assessing the distribution of prefrontal cortex thickness (excluding motor and premotor areas) in a large prospective, consecutive sample of patients with motor neuron disease.

2. Methods

2.1. Participants

In this prospective, cross-sectional study two centres enrolled $n = 158$ patients with motor neuron disease (site 1: $n = 45$, site 2: $n = 113$) between April 2011 and August 2014. In order to represent the whole spectrum of different motor neuron disease variants, the following phenotypes were included (Chio et al., 2011): classical ALS and bulbar phenotype ($n = 102$, "classical ALS"); primary lateral sclerosis ($n = 9$, "PLS"); upper motor neuron dominant ALS ($n = 13$, "UMN"); flail limb ($n = 10$) and progressive muscular atrophy ($n = 12$), merged to form the group lower motor neuron variants ("LMN"); ALS with comorbid frontotemporal dementia ($n = 13$, "ALS-FTD"). All patients were classified according to the revised El-Escorial criteria (Brooks et al., 2000) and physical disability was rated using the ALS functional rating scale revised (ALSFRS-R) (Cedarbaum et al., 1999). A concomitant diagnosis of frontotemporal dementia (FTD) was defined on clinical assessment in accordance with proposed diagnostic criteria and included interview of the accompanying caregiver. $n = 12$ patients fulfilled criteria for behavioural variant FTD (bvFTD) (Rascovsky et al., 2011); and $n = 1$ patient for non-fluent primary progressive aphasia (nfPPA) (Gorno-Tempini et al., 2011). Genetic testing was performed, looking for mutations in the superoxide dismutase 1 protein (*SOD1*), chromosome 9 open reading frame 72 protein (*C9orf72*), TAR DNA-binding protein 43 (*TARBDP*), ubiquilin protein (*UBQLN*), and RNA-binding protein fused in sarcoma (*FUS*). The identified genetic cases ($n = 16$) are listed in Table 1 and highlighted in Figs. 2 and 3. A general score of cognition was obtained using the Montreal Cognitive Assessment (MoCA) (Nasreddine et al., 2005), which was additionally adjusted in the patient cohort to control for motor disability (i.e. excluding subtests 1–3 from the general scoring). A group of 86 healthy controls was recruited (site 1: $n = 47$; site 2: $n = 39$) and clinically screened to exclude neurological illness and cognitive impairment (MoCA score ≥ 26). To assess general educational level, all participants completed a German version of the vocabulary test (WST) (Schmidt and Metzler, 1992). Demographic data of all participants are summarised in Table 1.

All participants underwent a detailed neuropsychological assessment, testing the domains of executive function, verbal memory, behaviour, and visuo-spatial skills. In order to make sure that cognitive impairment does not confound the data analysis, patients' performance on the following tests was utilised for additional classification into MND with cognitive impairment (MND-ci), MND with behavioural impairment (MND-bi), and MND with combined cognitive and behavioural impairment (MND-cbi) according to the revised Strong criteria (Strong et al., 2017): Letter fluency and flexibility indices, semantic fluency and flexibility indices, trail making test (TMT), Stroop test, backward digit span, and the apathy scale from the Frontal Systems Behaviour Scale (FrSBe). Patients were classified as MND-ci if they showed impairment in verbal fluency and/or flexibility or impairment on two other independent executive functions. Impairment was defined as scoring below 2 SD of the performance of the healthy controls in the corresponding test. To be categorised as MND-bi, patients had to be impaired in the apathy scale of the FrSBe. If patients fulfilled criteria for both MND-ci and MND-bi they were classified as MND-cbi. Out of 158 patients, $n = 25$ could not be classified due to missing neuropsychological assessment. Means and standard deviations of neuropsychological data are displayed in Supplementary Table 1.

The local ethics committees of both universities approved the study and all subjects gave written informed consent prior to their inclusion.

2.2. Data acquisition and analysis

High-resolution, T1-weighted structural MRI scans were acquired at both study sites using 3 T Siemens VERIO Magnetom scanners (Siemens, Erlangen/Germany) with an identical acquisition protocol that employed a 32-channel head coil and a 3D-MPRAGE sequence (echo time = 4.82 ms, repetition time = 2500 ms, inversion

Table 1
Demographic profile of participants.

	HC	MND	*p* value	Classical ALS	PLS	UMN	LMN	ALS-FTD	*p* value
No.	86	158		102	9	13	21	13	
Median age (range)	62.3 (33–83)	61.3 (32–83)	0.668[c]	60.3 (32–83)	62.7 (53–71)	57.3 (35–69)	64.6 (40–79)	66.7 (40–75)	0.087[b]
Sex [male (%)]	54 (62.8%)	98 (62.0%)	0.906[a]	61 (59.8%)	6 (66.7%)	9 (69.2%)	16 (76.2%)	6 (46.2%)	0.439[a]
Handedness [right (%)]	78 (90.7%)	149 (94.3%)	0.291[a]	96 (94.1%)	9 (100%)	13 (100%)	19 (90.5%)	12 (92.3%)	0.736[a]
Median ALSFRS-R (range)	–	39.0 (14–48)	–	39.0 (14–48)	36.0 (28–41)	38.0 (30–44)	41.0 (26–46)	41.0 (22–46)	0.060[b]
Median disease duration [months] (range)	–	16.6 (3.6–272.3)	–	15.8 (3.6–104.8)	93.4 (13.9–272.3)	11.7 (4.1–67.4)	18.6 (4.0–100.9)	15.1 (6.3–127.8)	0.003[b]
El Escorial (NA/possible/probable/definite)		23/44/32/59		4/22/25/51	0/8/1/0	0/8/1/4	17/3/1/0	2/3/4/4	–
SOD1 mutation (%)	–	5 (3.2%)	–	4 (3.9%)	0 (0%)	0 (0%)	1 (4.8%)	0 (0%)	–
C9orf72 repeat expansion (%)	–	11 (7.0%)	–	10 (9.8%)	0 (0%)	0 (0%)	0 (0%)	1 (7.7%)	–
Median WST (range)	32.0 (26–40); $n = 71$	30.0 (14–41); $n = 126$	< 0.001[c]	31.0 (14–41)	31.0 (18–39)	30.5 (15–33)	28.0 (18–35)	27.0 (17–37)	0.316[b]
Median MoCA (range)	27.0 (26–30); $n = 86$	25.5 (9–30); $n = 150$	< 0.001[c]	25.5 (12 − 30)	26.0 (21 − 30)	25.5 (18–30)	25.0 (21–30)	18.5 (9–24)	< 0.001[b]

HC: healthy controls; MND: motor neuron disease; PLS: primary lateral sclerosis; UMN: upper motor neuron variants; LMN: lower motor neuron variants; ALS-FTD: ALS with comorbid frontotemporal dementia; ALSFRS-R: ALS Functional Rating Scale revised (Cedarbaum et al., 1999); MoCA: Montreal Cognitive Assessment (Nasreddine et al., 2005).

[a] Based on Pearson's χ^2.

[b] Based on Kruskal-Wallis χ^2.

[c] Based on Wilcoxon U.

time = 1100 ms, flip angle = 7°, voxel size = 1 × 1 × 1 mm^3). Prior to this study, basic quality assurance tests were carried out using the American College of Radiation (ACR) phantom at both sites with no significant differences in the tested parameters. Data were analysed using FreeSurfer (Fischl, 2012) version 5.3.0 (http://surfer.nmr.mgh.harvard.edu/). Regional measures of cortical thickness were obtained from the automated anatomic parcellation (Desikan et al., 2006). Briefly, standardised preprocessing steps included intensity normalisation, skull stripping, Talairach transformation, and assignment of neuroanatomical labels to each voxel using the Desikan-Killiany probabilistic atlas (Desikan et al., 2006), resulting in 34 distinct cortical regions for each hemisphere. The obtained cortical segmentations were inspected for errors and no manual editing was necessary. The focus of the study was the prefrontal cortex, therefore we created a region of interest (ROI) of the prefrontal cortex by merging the following Desikan-Killiany regions of each hemisphere separately using mri_mergelabels as implemented in freesurfer: superior frontal gyrus, middle frontal gyrus (rostral division), inferior frontal gyrus (pars opercularis, pars orbitalis, pars triangularis), orbitofrontal cortex (lateral and medial division), frontal pole, and the anterior cingulate cortex (rostral and caudal division). Note that primary motor and premotor regions were not included in the prefrontal ROI. In order to demonstrate differences between motor and prefrontal regions, the caudal part of the bilateral middle frontal gyrus served as a ROI of the premotor cortex, whereas the bilateral precentral gyrus served as the ROI of the primary motor cortex. The occipital lobe was included as an additional ROI, as it is known to be the least affected cortical region by the ongoing motor degeneration, and therefore served as a reference region. It included the following Desikan-Killiany regions: lingual gyrus, pericalcerine cortex, cuneus cortex, and lateral occipital cortex. These regions were, similiar to the prefrontal ROI, merged using mri_mergelabels as implemented in FreeSurfer. Mean thickness values for the selected ROIs were obtained separately for each hemisphere and adjusted for age using the covariance method (Jack et al., 1989). No adjustment was done for the total intracranial volume (TIV) as it has been shown to be not related to cortical thickness (Barnes et al., 2010).

The distribution of cortical thickness values and demographic data was tested for normality of distribution using Shapiro-Wilk tests. Demographic variables were not normally distributed and differences between groups were assessed using chi-square (gender, handedness) and Kruskal-Wallis (age, WST, MoCA) or Wilcoxon tests (for group comparisons HC/MND). Differences in cortical thickness in each of the *a priori* defined ROIs were assessed for each hemisphere separately, conducting fixed effects models with group (HC/MND) and gender as fixed factors. In order to test a possible effect of scanning location, a random effect was added in a basic model (predicting cortical thickness values only by the intercept) by allowing the intercepts to vary across sites. For both models, the Akaike information criterion (AIC), Schwarz Bayesian criterion (BIC), and the log-likelihood of the chi-square likelihood ratio test were compared. Adding the scanning location did not improve the fit of the model (indicated by smaller AIC, BIC, and log-likelihood values) and was therefore not further considered in the analysis (Supplementary Table 2).

The statistical significance threshold was set to $p = 0.006$ (0.05/8) following Bonferroni correction to account for multiple comparison of 8 ROIs. Additionally, patients' and controls' cortical thickness values were *Z*-standardised by healthy control mean and standard deviation (SD) in each of the four ROIs to identify cases lying worse than −1 SD below healthy control mean using chi-square tests to assess differences in proportions between MND and HC.

In order to identify cortical thickness differences between clinical phenotypes, fixed effects models with phenotype (classical ALS/PLS/UMN/LMN) and gender as fixed factors were conducted. Note that ALS-FTD patients were excluded from this analysis to explore differences in thickness between varying degrees of upper and lower motor neuron involvement alone. The impact of cognitive and behavioural deficits on cortical thickness was tested using fixed effects models with cognitive phenotype (MND/MNDci, MNDbi, MND cbi/ALS-FTD) and gender as fixed factors. In case of a significant group effect, *post hoc* tests were performed by pairwise comparisons using *t*-tests with pooled standard deviation and results were corrected for multiple comparisons (false discovery rate (FDR); $p < 0.05$ was considered significant).

Spearman rank correlations were computed to determine the relationship between mean cortical thickness in all four ROIs and not-normally distributed clinical parameters, i.e. physical disability (ALSFRS-R), disease duration, and general cognitive ability (MoCA) with the significance level adjusted to $p = 0.017$ (0.05/3) following Bonferroni correction. The statistical analysis was done using R version 3.4.3.

3. Results

3.1. Group comparisons

Age-adjusted mean cortical thickness values in the left (HC: $W = 0.99$, $p = 0.91$; MND: $W = 0.98$, $p = 0.02$) and right (HC: $W = 0.98$, $p = 0.12$; MND: $W = 0.99$, $p = 0.19$) premotor, as well as in the left (HC: $W = 0.99$, $p = 0.84$; MND: $W = 0.98$, $p = 0.06$) and right (HC: $W = 0.99$, $p = 0.76$; MND: $W = 0.99$, $p = 0.26$) prefrontal and occipital (left HC: $W = 0.98$, $p = 0.18$; MND: $W = 0.99$, $p = 0.34$; right HC: $W = 0.98$, $p = 0.19$; MND: $W = 0.99$, $p = 0.54$) cortices were normally distributed in patients and controls (Fig. 1). Shapiro-Wilk test indicated a significant deviation of the left (HC: $W = 0.99$, $p = 0.79$; MND: $W = 0.96$, $p = 0.003$) and right (HC: $W = 0.95$, $p = 0.004$; MND: $W = 0.98$, $p = 0.02$) motor cortex thickness from the normal distribution in the patient cohort (and healthy controls for the left motor cortex), but inspection of the quantile-quantile (QQ) plots (Supplementary Fig. 1) and histograms (Fig. 1) did not show a major deviance.

Cortical thickness of the right ($p = 0.002$) motor cortex was significantly reduced in MND patients when compared to controls. Differences in left motor thickness did not reach the predetermined significance level ($p = 0.007$). Removing those patients with comorbid FTD (right: $p = 0.004$, left: $p = 0.02$) did not change these effects, as it was the case when removing those cases carrying a C9orf72 gene mutation (right: $p = 0.004$, left: $p = 0.01$). No between-group differences were found for the left ($p = 0.03$) and right prefrontal cortex ($p = 0.23$). There was no MND-related thinning in the premotor cortex (left: $p = 0.31$, right: $p = 0.13$) or occipital cortex (left: $p = 0.31$, right: $p = 0.16$). Means, standard deviations, and *F* values are summarised in Table 3.

Fig. 2 illustrates the distribution of mean cortical thickness values in patients when standardised to z-scores. In a normal distribution, 15.9% of cases are expected to fall outside −1 standard deviation. The proportion of MND patients falling outside −1 standard deviation of the control distribution was significantly increased for the right (29.1%, $p = 0.002$) motor cortical thickness (Table 2). A similar profile, although not reaching the significance level of $p = 0.006$, was observed for the left motor cortex (28.5%), right premotor cortex (27.8%), and left prefrontal cortex (26.6%) of MND cases lay worse than −1 SD from the healthy control mean, respectively, indicating that the patient cohort was shifted towards the left side of the control normal distribution. This was not the case for the occipital cortex.

3.2. Clinical phenotypes

There were no phenotype-related differences in cortical thickness values in the premotor, occipital, or prefrontal cortices (Table 3). Thinning of the left motor cortex was a function of clinical phenotype ($F = 2.63$, $p = 0.014$ (FDR-corrected)) in that PLS patients had worse cortical thinning than classical ALS patients and LMN variants (Supplementary Fig. 2).

With regard to cognitive phenotype (MND/MND-ci, MND-bi, MND-

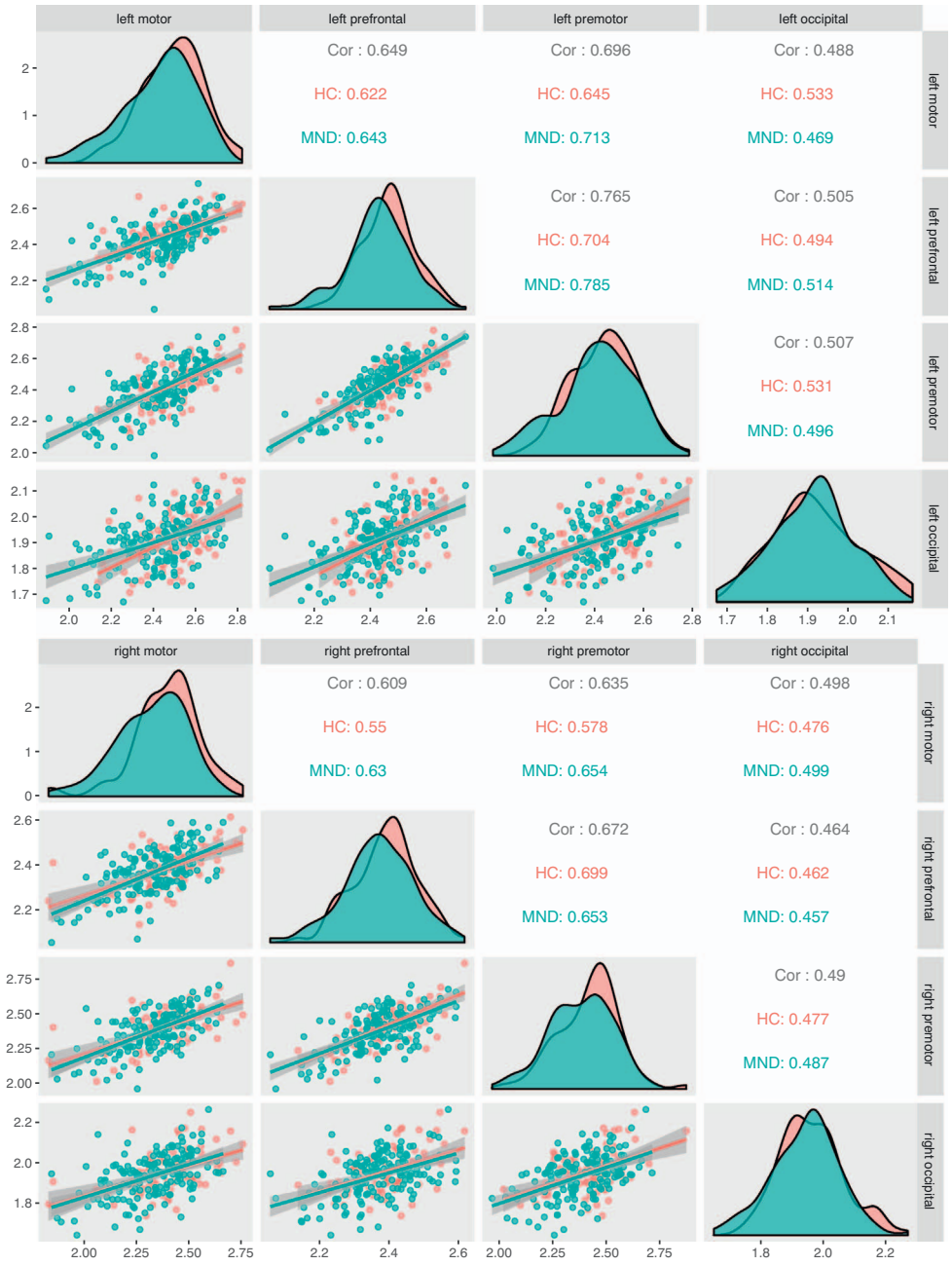

Fig. 1. Normal distribution (main diagonal) and inter-correlation (above the diagonal: Pearson correlation coefficients for MND (blue) and HC (red); below the diagonal: correlation scatterplots) of age-adjusted mean cortical thickness values (mm) for the motor cortex, premotor cortex, prefrontal cortex, and occipital cortex in healthy controls (red) and MND patients (blue). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

cbi/ALS-FTD), MND patients with cognitive and/or behavioural impairment compared to MND patients with no cognitive impairment had significantly thinner left motor and prefrontal cortices (Table 3). The distribution of z-standardised thickness values for the C9orf72 mutation carrier was comparable to the one of MND and MND-ci/bi patients, with exception of the right motor cortex, where distribution was similar to that of ALS-FTD patients, and the right occipital cortex (Supplementary Fig. 3).

3.3. Clinical correlations

Thinning of the left motor cortex was associated with lower ALSFRS-R scores ($\rho = 0.22$, $p = 0.006$) but not with disease duration ($\rho = -0.18$, $p = 0.022$). Cortical thickness values in the premotor, as well as in the prefrontal and occipital cortices were not associated with either physical disability or disease duration.

There was a significant relationship between patients' MoCA performance and prefrontal cortical thickness (left: $\rho = 0.34$, $p < 0.001$; right: $\rho = 0.28$, $p < 0.001$). This stayed significant when controlling the MoCA for motor impairment (left: $\rho = 0.32$, $p < 0.001$; right: $\rho = 0.27$, $p = 0.001$). A similar relationship was observed between patients' MoCA performance and thickness of the motor (left: $\rho = 0.35$, $p < 0.001$; right: $\rho = 0.34$, $p < 0.001$) and premotor cortices (left: $\rho = 0.33$, $p < 0.001$; right: $\rho = 0.24$, $p = 0.003$) (Fig. 3). Thickness of the left occipital cortex was also associated with patients' MoCA performance, although to a lower degree ($\rho = 0.24$, $p = 0.003$).

After removing those patients with comorbid FTD, the relationship between MoCA performance and motor cortical thickness remained significant (left: $\rho = 0.22$, $p = 0.009$; right: $\rho = 0.24$, $p = 0.004$). A similar relationship was found when removing C9orf72 mutation carriers

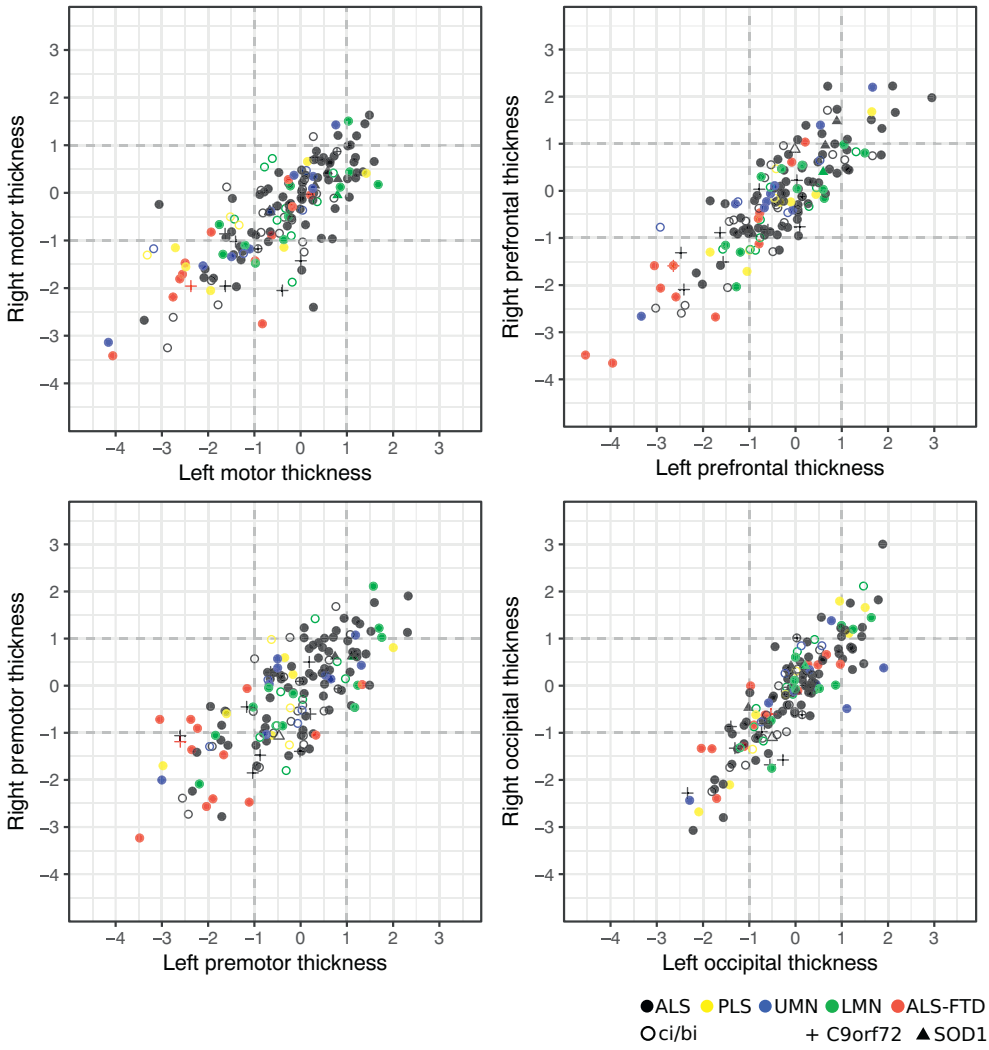

Fig. 2. Distribution of age-adjusted, z-standardised mean cortical thickness values for left and right motor cortex, premotor cortex, prefrontal cortex, and occipital cortex for classical ALS (black), PLS (yellow), UMN (blue), LMN (green), and ALS-FTD (red). Crosses indicate C9orf72 repeat expansion carriers (+), and triangles indicate SOD1 mutation carriers (Δ). Unfilled symbols indicate patients with comorbid cognitive (MND-ci) or behavioural (MND-bi) impairment. *Z*-values are standardised by healthy control mean and standard deviation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(left: ρ = 0.22, p = 0.009; right: ρ = 0.24, p = 0.004). This was not the case for the premotor (left: ρ = 0.13, p = 0.122; right: ρ = 0.08, p = 0.325), prefrontal (left: ρ = 0.18, p = 0.036; right: ρ = 0.14, p = 0.092), and occipital cortices (left: ρ = 0.14, p = 0.103; right: ρ = 0.06, p = 0.509). There was no relationship between healthy controls' MoCA performance and cortical thickness of the motor and premotor as well as the occipital and prefrontal cortices.

4. Discussion

The results of this prospective, cross-sectional study indicate an increased prevalence of prefrontal cortical thinning in patients with motor neuron disease and cognitive or behavioural deficits. Our data indicate that the distribution of prefrontal thickness was unimodal and independent of motor neuron disease phenotype, disease duration or

Table 2
Incidence of cases lying 1 SD below/above healthy control mean cortical thickness[a].

Cortical thickness		MND < M−1SD (%)	HC < M−1SD (%)	χ^2	p value	MND > M + 1SD (%)	HC > M + 1SD (%)	χ^2	p value
Motor cortex	Left	45 (28.5%)	15 (17.4%)	3.66	0.06	17 (10.8%)	12 (14.0%)	0.54	0.46
	Right	46 (29.1%)	10 (11.6%)	9.63	**0.002**	8 (5.1%)	9 (10.5%)	2.51	0.11
Premotor cortex	Left	34 (21.5%)	16 (18.6%)	0.29	0.59	27 (17.1%)	14 (16.3%)	0.03	0.87
	Right	44 (27.8%)	16 (18.6%)	2.57	0.12	20 (12.7%)	10 (11.6%)	0.05	0.82
Prefrontal cortex	Left	42 (26.6%)	16 (18.6%)	1.96	0.16	18 (11.4%)	15 (17.4%)	1.74	0.19
	Right	31(19.6%)	15 (17.4%)	0.17	0.68	17 (10.8%)	15 (17.4%)	2.18	0.14
Occipital cortex	Left	27 (17.1%)	12 (14.0%)	0.41	0.52	19 (12.0%)	16 (18.6%)	1.96	0.16
	Right	35 (22.2%)	12 (14.0%)	2.41	0.12	18 (11.4%)	12 (14.0%)	0.34	0.56

M: mean of the healthy controls; SD: standard deviation.
Significant values are shown in bold ($p \leq 0.006$, following Bonferroni correction).
[a] In a normal distribution, 15.9% of cases are expected to fall outside ± 1 SD, 6.7% outside ± 1.5 SD, and 2.5% outside ± 2 SD.

Table 3
Means and standard deviations of cortical thickness values for different clinical phenotypes.

	Motor cortical thickness		Premotor cortical thickness		Prefrontal cortical thickness		Occipital cortical thickness	
	Left	Right	Left	Right	Left	Right	Left	Right
HC	2.50 ± 0.15	2.40 ± 0.16	2.44 ± 0.13	2.42 ± 0.14	2.46 ± 0.09	2.39 ± 0.09	1.92 ± 0.11	1.96 ± 0.10
MND	2.43 ± 0.18	2.33 ± 0.17	2.41 ± 0.16	2.38 ± 0.15	2.43 ± 0.11	2.37 ± 0.10	1.91 ± 0.10	1.94 ± 0.11
F	5.03	6.57	1.81	2.08	3.69	1.47	1.17	1.87
p	**0.007**	**0.002**	0.309	0.128	0.026	0.232	0.311	0.156
class. ALS	2.46 ± 0.15	2.35 ± 0.16	2.43 ± 0.14	2.40 ± 0.14	2.44 ± 0.10	2.38 ± 0.09	1.91 ± 0.10	1.94 ± 0.11
PLS	2.30 ± 0.22	2.27 ± 0.14	2.37 ± 0.17	2.38 ± 0.14	2.43 ± 0.09	2.36 ± 0.09	1.90 ± 0.13	1.94 ± 0.17
UMN	2.36 ± 0.21	2.30 ± 0.19	2.39 ± 0.16	2.39 ± 0.12	2.40 ± 0.13	2.38 ± 0.10	1.94 ± 0.11	1.96 ± 0.10
LMN	2.47 ± 0.14	2.35 ± 0.13	2.45 ± 0.15	2.40 ± 0.15	2.45 ± 0.09	2.37 ± 0.08	1.94 ± 0.08	1.97 ± 0.10
F	3.88	2.21	1.14	1.19	0.95	0.40	1.80	1.86
p	**0.005**	0.071	0.338	0.317	0.440	0.808	0.132	0.122
	ALS vs. PLS $p = 0.032$							
	LMN vs. PLS $p = 0.032$							
MND	2.46 ± 0.17	2.35 ± 0.16	2.44 ± 0.15	2.41 ± 0.02	2.45 ± 0.10	2.39 ± 0.09	1.92 ± 0.10	1.95 ± 0.11
MNDci/bi	2.40 ± 0.15	2.31 ± 0.07	2.39 ± 0.11	2.36 ± 0.14	2.41 ± 0.10	2.36 ± 0.09	1.90 ± 0.07	1.94 ± 0.09
ALS-FTD	2.26 ± 0.19	2.17 ± 0.17	2.21 ± 0.17	2.22 ± 0.14	2.28 ± 0.14	2.26 ± 0.13	1.86 ± 0.11	1.91 ± 0.09
F	6.65	6.55	9.98	8.53	11.32	8.08	2.28	1.57
p	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	0.081	0.199
	MND vs. MNDci/bi $p = 0.042$	MNDci/bi vs. ALS-FTD $p = 0.009$	MNDci/bi vs. ALS-FTD $p \leq 0.001$	MNDci/bi vs. ALS-FTD $p = 0.004$	MND vs. MNDci/bi $p = 0.048$	MNDci/bi vs. ALS-FTD $p = 0.002$		
	MNDci/bi vs. ALS-FTD $p = 0.016$	MND vs. ALS-FTD $p \leq 0.001$	MND vs. ALS-FTD $p \leq 0.001$	MND vs. ALS-FTD $p \leq 0.001$	MNDci/bi vs. ALS-FTD $p < 0.001$	MND vs. ALS-FTD $p \leq 0.001$		
	MND vs. ALS-FTD $p \leq 0.001$				MND vs. ALS-FTD $p \leq 0.001$			

Significant values are shown in bold ($p \leq 0.006$, following Bonferroni correction).

severity, but associated with cognitive or behavioural impairment.

MND-related thinning was observed in the right motor cortex, whereas thickness in the prefrontal, premotor, and occipital cortex did not differ between groups when subgroups were not considered. Differences in motor cortical thickness remained significant even after removing those patients with comorbid FTD and C9orf72 mutation carriers, indicating that motor cortical thickness is reduced throughout the MND spectrum. Although cortical thickness values were normally distributed, the proportion of MND cases lying worse than −1 SD below the control mean in the right motor cortex was significantly higher than would be expected by chance, indicating a shift of distribution towards thinner cortex in this region (Table 2). A similar pattern was observed in the left motor, left prefrontal, and right premotor cortex, although not reaching the predetermined significance level.

Thinning of the left motor cortex tended to be a function of clinical phenotype in that PLS variants had worse cortical thinning than classical ALS patients and LMN variants. This is in line with recent studies on cortical thickness in the motor cortex, where thinning is reported to be highest in UMN variants (of which PLS presents an extreme), followed by classical ALS, while pure LMN variants do not differ from disease mimics (Walhout et al., 2015b) and healthy controls (Schuster et al., 2013). Notably, this phenotype specific pattern was not observed in the premotor, prefrontal, and occipital cortices.

When segregating the MND patients based on their cognitive performance, a significant shift towards thinner motor and prefrontal cortices was identified in patients with cognitive and/or behavioural impairment when compared to MND patients without cognitive dysfunction. Although left prefrontal thickness values differed between these groups, the overlaying kernel density estimations (Supplementary Fig. 3) showed a similar distribution while shifted towards thinner cortex in MND with cognitive impairment, as it was the case for ALS patients with comorbid FTD. This was supported by the fact that ALS patients with comorbid FTD were more likely to be worse than −1 SD in the prefrontal cortex, but not all of them showed prefrontal cortical thinning. The majority of ALS-FTD patients included in this study (12 out of 13) fulfilled the criteria for bvFTD (Rascovsky et al., 2011), where behavioural changes are the hallmark of the disease and prefrontal cortical atrophy is a common feature. Nevertheless, heterogeneity in atrophy patterns across bvFTD is high and can depend on patients' behavioural profile (Massimo et al., 2009; Walhout et al., 2015a), genotype (Whitwell et al., 2012), or subtype (Whitwell et al., 2009). Within this scenario, it could also be possible, that a lot of different subtypes within the established phenotypes led to the relatively uniform distribution. Further studies with larger sample sizes accounting for the large heterogeneity in phenotypes have to be conducted in order to exclude this possible confound.

The identified thinning of the prefrontal and motor cortex was specific for MND with cognitive and/or behavioural impairment in that no cortical thinning was observed in the occipital control region or the premotor cortices, showing that the observed thinning is not indicative of a global atrophy process. The occipital lobe was thought to be relatively spared from the ongoing neurodegeneration associated with MND, but recent studies show that especially C9orf72 mutation carrier can show involvement of occipital regions in symptomatic (Westeneng et al., 2016) and even asymptomatic disease stages (Walhout et al., 2015a). Interestingly, although the numbers are too small to draw any further conclusions, our results indicate a shift towards thinner right occipital cortex for the included C9orf72 population but not for MND, MND with cognitive/behavioural deficits, or even ALS-FTD (Supplementary Fig. 3).

The clinical impact of frontal lobe thinning is further highlighted by the identified relationship between cortical thinning in the bilateral prefrontal, premotor, and motor areas and patients' general cognitive abilities. Although it is now recognised that frontal lobe dysfunction in MND encompasses more than executive dysfunction (Abrahams, 2013;

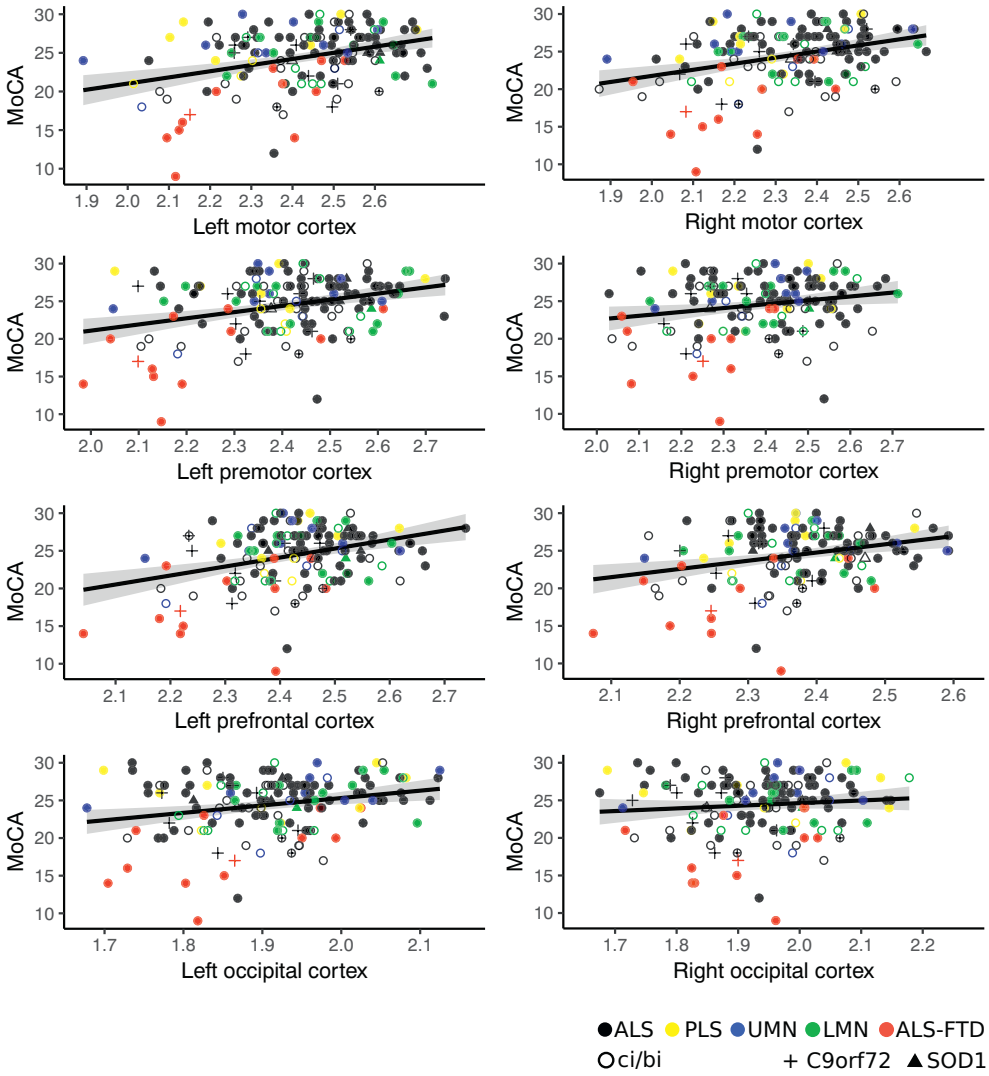

Fig. 3. Correlation of patients' motor, premotor, and prefrontal cortical thickness with general cognitive ability (MoCA) for classical ALS (black), PLS (yellow), UMN (blue), LMN (green), and ALS-FTD (red). Crosses indicate C9orf72 repeat expansion carriers (+), and triangles indicate SOD1 mutation carriers (Δ). Unfilled symbols indicate patients with comorbid cognitive (MND-ci) or behavioural (MND-bi) impairment. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Canu et al., 2013; Raaphorst et al., 2011), the MoCA as a measure of general cognitive impairment seems to be a valid instrument to map these changes. Interestingly, it was not only associated with prefrontal lobe thinning but also premotor and motor thinning in the patient cohort. Movement and cognition are closely interrelated, and the premotor cortex, in particular, plays an important role in mediating input information from the prefrontal cortex to the motor cortex (Dum and Strick, 1991). Therefore, it is not surprising that thinning in all of these regions was related to the patients' decline in general cognitive abilities. This relationship was not observed in the healthy control group, although it must be considered that, according to the inclusion criteria, they were not allowed to score below the cut-off of 26 points in the MoCA, resulting in a rather small range within this group that could have biased the results.

In summary, this study demonstrated that the prevalence of prefrontal cortical thinning is increased in MND phenotypes and related to patients' general cognitive ability. Prefrontal cortical thickness was normally distributed in MND but shifted towards thinner cortex in MND with cognitive and/or behavioural impairment compared to MND patients without cognitive impairment, suggesting a more general vulnerability to FTD for this group. There was also evidence that, with primary motor and premotor regions omitted, prefrontal cortical thinning is independent of the degree of upper and lower motor neuron involvement, disease duration, and physical disability, but related to cognitive dysfunction.

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Competing interests

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