Upper motor and extra-motor neuron involvement in recent-onset motor neuron disease
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Upper and extra-motor neuron involvement in early motor neuron disease: a diffusion tensor imaging study

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ABSTRACT

Background
Motor neuron disease is a term encompassing three phenotypes defined largely by the balance of upper versus lower motor neuron involvement, namely amyotrophic lateral sclerosis, primary lateral sclerosis and progressive muscular atrophy. However, neuroradiological and pathological findings in these phenotypes suggest that degeneration may exceed the neuronal system upon which clinical diagnosis is based.

Methods
To further delineate the phenotypes within the motor neuron disease spectrum, this controlled study assessed the upper motor and extra-motor neuron white matter involvement in cohorts of patients with motor neuron disease phenotypes shortly after diagnosis by comparing diffusion tensor imaging data of the different cohorts to those of healthy controls and directly between the motor neuron disease phenotypes (n = 12 for each cohort). Furthermore, we acquired follow-up data 6 months later to evaluate fractional anisotropy changes over time. Combined use of diffusion tensor tractography of the corticospinal tract and whole-brain voxel-based analysis allowed for the comparison of the sensitivity of these techniques to detect white matter involvement in motor neuron disease.

Results
The voxel-based analysis demonstrated varying extents of white matter involvement in different phenotypes of motor neuron disease, albeit in quite similar anatomical locations. In general, fractional anisotropy reductions were modest in progressive muscular atrophy and most extensive in primary lateral sclerosis. The most extensive patterns of fractional anisotropy reduction were observed over time in the voxel-based analysis, indicating progressive extra-motor white matter degeneration in limb- and bulbar onset amyotrophic lateral sclerosis and in progressive muscular atrophy.

Conclusion
The observation of both upper motor and extra-motor neuron involvement in all phenotypes of motor neuron disease shortly after diagnosis suggests that these are all part of a single spectrum of multisystem neurodegenerative disease. Voxel-based analysis was more sensitive to detect longitudinal changes than diffusion tensor tractography of the corticospinal tract. Voxel-based analysis may be particularly valuable
in the evaluation of motor and extra-motor white matter involvement in the early symptomatic stages of motor neuron disease, and for monitoring the spread of pathology over time.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease, characterized by progressive upper and lower motor neuron signs. The primary target of disease remains unknown.\(^1\)-\(^3\) Progressive muscular atrophy (PMA) is defined by progressive lower motor neuron signs and diagnosed after exclusion of other lower motor neuron syndromes.\(^4\) Its prognosis resembles that of ALS and upper motor neuron signs may occur in the course of the disease.\(^5\)-\(^8\) Primary lateral sclerosis (PLS) is a pure upper motor neuron syndrome of slowly progressive, usually spinobulbar, spasticity and is typically associated with a much longer survival.\(^9\),\(^10\)

The exact relationship between PMA, ALS and PLS remains unresolved. ALS may well encompass various phenotypes with different aetiologies or different modifying factors.\(^9\),\(^11\),\(^12\) Although the cardinal sign of ALS is motor impairment, it has become clear that ALS is a multisystem disease, in which widespread extra-motor brain pathology can be found.\(^13\)-\(^18\)

Diffusion tensor imaging is able to characterize the diffusion properties of water molecules \textit{in vivo}.\(^19\) This diffusion is restricted or hindered by the presence of barriers, such as cellular membranes and subcellular structures, which behave as obstacles to the free motion of water. As a result, the water molecules tend to diffuse preferentially in orientations relatively free of obstruction, such as along axons, leading to a directional bias or anisotropic diffusion.\(^20\) Therefore, changes in anisotropy, which can be quantified by diffusion tensor imaging (for instance, by assessing the fractional anisotropy), reflect changes in tissue microstructure and organization of fibres.\(^21\) In particular, it has been suggested that a reduction in anisotropy can reflect axonal fiber degeneration and myelin breakdown in both the peripheral and central nervous system.\(^22\),\(^23\)

With diffusion tensor tractography it is possible to reconstruct specific major white matter tracts such as the corticospinal tract.\(^24\) When extending the focus beyond a specific white
Matter tract, whole-brain voxel-based analysis can be applied to assess the whole-brain white matter in a single comparison.18, 25-27

Previous diffusion tensor imaging studies found decreased fractional anisotropy to be related to clinical18 and electrophysiological26, 28 measures of upper motor neuron degeneration in patients with ALS, indicating that diffusion tensor imaging has potential in the objective evaluation of upper motor neuron degeneration in ALS. However, most studies only included typical patients with ALS, with few studies investigating different motor neuron disease phenotypes concomitantly29-31 or longitudinally32-34. Also, most studies included patients with heterogeneous disease duration, and none of these studies were aimed specifically at investigating patients shortly after diagnosis.

To further delineate the various phenotypes (ALS, PLS and PMA) within the motor neuron disease spectrum with respect to the profile of upper- and extra-motor white matter involvement, this controlled study assessed the white matter involvement in cohorts of patients with motor neuron disease phenotypes shortly after diagnosis by comparing diffusion tensor imaging data of the different cohorts to those of healthy controls and directly between the phenotypes. Furthermore, we acquired follow-up data 6 months after the first scan session in order to evaluate fractional anisotropy changes over time. Combined use of diffusion tensor tractography and voxel-based analysis allowed comparison of the sensitivity of these techniques to detect white matter involvement in motor neuron disease.

MATERIALS AND METHODS

Subjects
We included cohorts of patients with bulbar-onset ALS (ALS-B), limb-onset ALS (ALS-L), PMA, PLS, and healthy controls (n=12 for each cohort). Patients with ALS and PMA were recruited from tertiary referral neuromuscular clinics in The Netherlands (University Medical Centres of Amsterdam, Utrecht and Rotterdam) and the Catharina Hospital in Eindhoven. All examinations upon inclusion and at follow-up were performed by the same investigator (M.M.v.d.G.).

All patients with ALS met the revised El Escorial criteria for probable, probable laboratory-supported or definite ALS.35
PMA was diagnosed when clinical and electrophysiological evidence of progressive pure lower motor neuron involvement was present in two or more regions, excluding patients with focal or segmental muscular atrophy, and after ruling out other lower motor neuron diseases by extensive neurophysiological testing and neuroimaging if necessary. All patients had weakness (i.e. disease duration) for < 1 year, except for the patients with PLS who, by definition, had a longer disease duration.

Nine out of the 12 patients with PLS were recruited from a cohort study which included patients with an apparently sporadic, adult-onset idiopathic upper motor neuron syndrome at the University Medical Centre in Utrecht. In this study, one of the inclusion criteria was a disease duration of at least 3 years, which is shorter than the definition currently in use (disease duration at least 4 years) and alternative diagnoses had been ruled out by extensive neurophysiological and genetic testing. The remaining patients with PLS were recruited from the other tertiary referral neuromuscular outpatient departments.

Healthy age-matched controls were recruited from hospital personnel and partners of patients. Follow-up visit for all cohorts, except PLS, was 6 months after baseline. PLS patients only provided baseline data, meant to obtain reference values for longstanding and prominent upper motor neuron involvement.

The study was approved by the local ethical committee and all subjects gave written informed consent.

Clinical parameters
We measured finger tapping speed (number of taps per 10 seconds, subsequently expressed as taps/second after averaging values of the left and right side), as deceleration of tapping speed suggests an upper motor neuron lesion. Vital capacity was measured with a handheld spirometer and expressed as a percentage of expected normal value. The revised ALS functional rating scale (ALSFRS-R, scores ranging 0-48) was administered to all subjects. Furthermore, we calculated the disease progression rate as follows: disease progression rate = (48 – ALSFRS-R score)/disease duration.

Data acquisition
All diffusion tensor imaging data were acquired on a single 3 Tesla MRI system (Philips Intera, Philips Medical Systems, Best, The Netherlands) in the Academic Medical Centre in Amsterdam, The Netherlands, using a spin-echo echo-planar imaging sequence.
The diffusion-weighting was performed along 32 directions, with a b-value of 1000 s/mm². Additionally, one set of images was acquired without diffusion-weighting (b = 0 s/mm²). Other sequence parameters were: 64 contiguous axial slices, echo time = 94 ms, repetition time = 8115 ms, field-of-view = 250 mm, scan matrix = 109 x 112, interpolated image matrix = 256 x 256, slice thickness = 2.2 mm, non-interpolated voxel size = 2.2 x 2.2 x 2.2 mm³. Diffusion tensor imaging scan time was ~ 8 minutes.

Data processing
The raw diffusion-weighted images were motion and eddy current corrected using a linear affine registration in the phase direction and the images were filtered for noise using the Linear Minimum Mean Square Estimator. Additionally, the b-matrix was corrected for the rotational component of the motion correction to ensure that errors in the diffusion-weighting that originate from these rotations could be minimized. The diffusion tensor was estimated from the corrected diffusion-weighted images and from this fractional anisotropy was derived.

Diffusion tensor tractography: fibre tracking and spatial profiling along the corticospinal tract
The corticospinal tract in the brain was reconstructed bilaterally in each subject using diffusion tensor imaging studio software, which uses the Fibre Assignment by Continuous Tracking method. We set a fractional anisotropy-threshold of 0.2 and an angular threshold of 50° for all reconstructions. Regions of interest and algorithm settings were defined in accordance with previous reports on fibre tracking of the corticospinal tract.

Diffusion tensor tractography is known to be a user-dependent process. Therefore, fibre tracking reproducibility was assessed by two blinded observers (M.M.v.d.G., C.S.) reconstructing the corticospinal tract bilaterally in a subset of 10 subjects, randomly chosen from the entire study population. The overlapping tract volume was computed relative to the total volume tracked by both observers.

Subsequently, one blinded observer (M.M.v.d.G.) performed the region of interest definition for bilateral corticospinal tract reconstruction in all subjects. For individuals who completed the study, i.e. with diffusion tensor imaging data at two time points, scans of both time points were matched using a rigid transformation of the fractional anisotropy maps as implemented in SPM5 (Statistical Parametric Mapping, Wellcome Department.
of Imaging Neuroscience, University College London) so that regions of interest had to be defined only once for each subject.

From the diffusion tensor tractography reconstructions, we first computed mean fractional anisotropy values per tract, thus yielding two values (left and right side) per patient per time point. Second, we generated fractional anisotropy profiles along the caudocranial course of the corticospinal tract as follows: we coregistered the fractional anisotropy maps to the ICBM-81 fractional anisotropy template using joint affine and non-rigid registration using smooth basis functions. By applying these transformations to the voxel label maps (i.e. binary images of the corticospinal tract reconstructions, with voxels having a value of 1 being part of the diffusion tensor tractography reconstruction), the tract data of all subjects resides in one common reference frame. To increase the signal-to-noise ratio and reduce the number of data points these datasets were then resampled at a resolution of 4 mm, yielding fractional anisotropy profiles sampled at 23 positions in a caudocranial direction for both left and right corticospinal tract of all individual subjects. In case diffusion tensor tractography terminated in the pons, we extended tracking in these datasets by the population tracking template, which included all voxels that were non-zero in > 50% of the subjects’ non-rigidly transformed label maps.

Voxel-based analysis: whole-brain white matter assessment
The diffusion tensor imaging data post-processing for voxel-based analysis was identical to the protocol described in Sage et al., in which we demonstrated that by using improved coregistration and a population-based diffusion tensor imaging atlas for the processing of diffusion tensor imaging data, the reliability of voxel-based analysis can be improved. This ‘optimized’ voxel-based analysis yielded similar results as those obtained by tract-based spatial statistics. In short, data processing included coregistering all diffusion tensor imaging datasets non-rigidly to a population-based diffusion tensor imaging atlas, which was generated from the diffusion tensor imaging data from all subjects included in this study. Finally, the warped fractional anisotropy maps were anisotropically smoothed, as recommended in a very recent paper. This type of smoothing preserves the boundaries and sharpens edges between structures while removing noise in homogeneous regions.

Statistical analysis
For all tests applied to clinical parameters and fractional anisotropy data in the diffusion...
tensor tractography study, we set a threshold for statistical significance of $p < 0.05$ uncorrected. For all tests applied in the voxel-based analysis study, we set a threshold for statistical significance of $p < 0.001$ uncorrected.

Clinical data
We compared the clinical data of the various patient groups with those of other patient groups and those of controls using a Mann-Whitney U test for non-parametric data. Longitudinal data in each cohort were analyzed with the Wilcoxon signed ranks test for paired data.

Diffusion tensor tractography
Before pooling values of both hemispheres for further analyses, we compared mean fractional anisotropy of the corticospinal tract corresponding with the clinical site of disease onset (if applicable) with the contralateral hemisphere fractional anisotropy using the Wilcoxon signed ranks test for paired data.
We compared mean fractional anisotropy at baseline and the fractional anisotropy profiles at baseline along the corticospinal tract between the various patient groups and between patients and controls, using a Mann-Whitney U test.
Finally, we compared mean fractional anisotropy and fractional anisotropy profiles along the corticospinal tract obtained at baseline and at follow-up in all groups (except for PLS) using the Wilcoxon signed rank test for paired data.

Voxel-based analysis
Non-parametric statistical testing was performed using the Statistical non-Parametric Mapping toolbox available for SPM5 (Statistical Parametric Mapping, University College London). Fractional anisotropy maps of each patient group were compared with those of healthy controls with non-parametric two-sample $t$-tests, yielding four comparisons of patients versus controls (ALS-L/ALS-B/PLS/PMA at baseline versus controls at baseline).
Furthermore, we also performed direct comparisons between the different motor neuron disease phenotypes at baseline using non-parametric two-sample $t$-tests, yielding six comparisons between patient groups.
Finally, we assessed fractional anisotropy changes over time in all cohorts, except for PLS by performing non-parametric paired $t$-tests in the Statistical non-Parametric Mapping toolbox.
**Power analysis**

Based on a previous study, to detect a 5% decrease of fractional anisotropy in patients when compared to controls with a power of 80% and an alpha of 1%, 12 individuals per cohort were needed.\(^4\)

**RESULTS**

At follow-up, 7 out of 12 patients with ALS-L, 9 out of 12 patients with ALS-B, 10 out of 12 patients with PMA patients and 12 controls were able to undergo a repeated scan session. Reasons for lost-to-follow-up include 1) death (3 patients with ALS-L and 2 patients with PMA), 2) too disabled to visit the hospital (1 patient with ALS-L and 2 patients with ALS-B) and 3) inability to lie in supine position (1 patient with ALS-L and 1 patient with ALS-B). The baseline and follow-up data of one control subject had to be discarded due to imaging artifacts.

**Table 1. Baseline data of four cohorts of MND phenotypes and healthy controls**

<table>
<thead>
<tr>
<th></th>
<th>PLS (n=12)</th>
<th>ALS-B (n=12)</th>
<th>ALS-L (n=12)</th>
<th>PMA (n=12)</th>
<th>Controls (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M:F</td>
<td>7:5</td>
<td>5:7</td>
<td>10:2</td>
<td>11:1</td>
<td>7:5</td>
</tr>
<tr>
<td>(^1) patients with bulbar signs (n)</td>
<td>8</td>
<td>12</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>(^2) revised El Escorial criteria a/b/c</td>
<td>NA</td>
<td>2/8/2</td>
<td>1/10/1</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Age, years (median/range)</td>
<td>59.0 (45-74)</td>
<td>56.5 (42-72)</td>
<td>58.0 (41-78)</td>
<td>60.5 (44-72)</td>
<td>56.5 (40-85)</td>
</tr>
<tr>
<td>duration symptoms, months</td>
<td>50.2±24.8</td>
<td>9.8±3.2</td>
<td>10.5±2.5</td>
<td>10.8±2.0</td>
<td>NA</td>
</tr>
<tr>
<td>diagnostic delay (months)</td>
<td>24.4±16.0</td>
<td>6.7±2.7</td>
<td>6.6±2.5</td>
<td>6.6±2.9</td>
<td>NA</td>
</tr>
<tr>
<td>disease progression rate</td>
<td>0.010±0.005</td>
<td>0.026±0.018</td>
<td>0.027±0.016</td>
<td>0.020±0.010</td>
<td>NA</td>
</tr>
<tr>
<td>vital capacity, %</td>
<td>91.5±23.3(^a)</td>
<td>98.5±17.9(^b)</td>
<td>101.3±23.8</td>
<td>102.5±14.9</td>
<td>114.6±18.5</td>
</tr>
<tr>
<td>ALSFRS-R</td>
<td>35.0±6.6(^c)</td>
<td>40.1±4.7(^a)</td>
<td>40.1±3.9(^b)</td>
<td>41.8±3.7(^c)</td>
<td>48.0±5.0</td>
</tr>
<tr>
<td>finger tapping/s</td>
<td>2.3±1.2 (^d)</td>
<td>4.0±1.3 (^a)</td>
<td>3.3±1.3 (^b)</td>
<td>4.8±0.7</td>
<td>5.1±0.6</td>
</tr>
<tr>
<td>mean FA CST</td>
<td>0.42±0.02 (^e)</td>
<td>0.42±0.03 (^a)</td>
<td>0.43±0.03</td>
<td>0.46±0.02</td>
<td>0.45±0.02</td>
</tr>
</tbody>
</table>

**Abbreviations:** MND, motor neuron disease; PLS, primary lateral sclerosis; ALS-L, limb onset ALS; ALS-B, bulbar onset ALS; PMA, progressive muscular atrophy; M, male; F, female; ALSFRS-R, revised ALS functional rating scale; FA, fractional anisotropy; CST, corticospinal tract; NA, not applicable

Data are given as mean ± standard deviation, unless otherwise indicated. Statistical significance based on Mann Whitney U test comparing patient groups with controls, threshold set at p<0.05 (p-values in superscript).

\(^1\) defined as a score of 3 or lower on at least two of the three bulbar items of the ALSFRS-R (speech, saliorthoea, swallowing) at baseline

\(^2\) a: probable/ b: probable laboratory supported/c: definite

\(^3\) disease progression rate = (48-ALSFRS-R score at baseline)/disease duration (days)

\(^4\) finger tapping = (L+R)/2
Clinical characteristics at baseline and at follow-up

Baseline characteristics of all groups and significant differences between patient groups and controls are summarized in Table 1. Three out of the twelve patients with PLS had a disease duration of < 3 year (19-28 months) upon inclusion. These patients were re-examined 1.5 year after the baseline scan. None of them had developed lower motor neuron symptoms, vital capacity had remained stable and ALSFRS-R score reduction was < 2 points. Thus, the clinical picture was still compatible with a slowly progressive upper motor neuron syndrome. When comparing the different patient groups, disease progression rate at baseline was significantly slower in PLS than in ALS-L ($p = 0.003$), ALS-B ($p = 0.013$), and PMA ($p = 0.009$). Vital capacity at baseline did not differ significantly between any of the patient groups, but was significantly lower in ALS-B ($p = 0.043$) and PLS ($p = 0.028$) when compared with controls. The ALSFRS-R score was significantly lower in PLS than in ALS-L ($p = 0.010$), ALS-B ($p = 0.009$) and PMA ($p = 0.001$) and in all MND phenotypes when compared with controls ($p < 0.001$ for all comparisons). Finger tapping speed was significantly lower in PLS than in ALS-B ($p = 0.004$), PMA ($p < 0.001$) and controls ($p < 0.001$), significantly lower in ALS-L than in PMA ($p = 0.006$) and controls ($p = 0.002$), and significantly lower in ALS-B than in controls ($p = 0.013$). Disease progression rate, vital capacity and ALSFRS-R score did not differ significantly between PMA and ALS-L or ALS-B, or between ALS-L and ALS-B.

Table 2: Clinical characteristics and mean FA: changes over time (6 months)

<table>
<thead>
<tr>
<th></th>
<th>ALS-B (n=9)</th>
<th>ALS-L (n=7)</th>
<th>PMA (n=10)</th>
<th>Controls (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>revised El Escorial criteria</td>
<td>0/7/2</td>
<td>1/6/0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>patients with bulbar signs (n)</td>
<td>9</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>vital capacity (%)</td>
<td>97.7 ±15.7 (±SD)</td>
<td>106.9 ±19.3</td>
<td>101.0 ±16.0 (±SD)</td>
<td>115.6 ±19.0</td>
</tr>
<tr>
<td>ALSFRS-R</td>
<td>41.7 ±3.4 (±SD)</td>
<td>42.6 ±2.0 (±SD)</td>
<td>41.8 ±3.5 (±SD)</td>
<td>48.0 ±0.0</td>
</tr>
<tr>
<td>finger tapping/s</td>
<td>4.2 ±1.2 (±SD)</td>
<td>3.3 ±1.0 (±SD)</td>
<td>4.8 ±0.8</td>
<td>4.9 ±0.9</td>
</tr>
<tr>
<td>Mean FA CST</td>
<td>0.43 ±0.0 (±SD)</td>
<td>0.44 ±0.0 (±SD)</td>
<td>0.46 ±0.02</td>
<td>0.45 ±0.02</td>
</tr>
</tbody>
</table>

Abbreviations: ALS-B, bulbar onset ALS; ALS-L, limb onset ALS; PMA, progressive muscular atrophy; ALSFRS-R, revised ALS functional rating scale; FA, fractional anisotropy; CST, corticospinal tract.

Data are given as mean ± standard deviation, unless otherwise indicated. Statistical significance based on Wilcoxon test for paired data, threshold set at $p<0.05$ ($p$-values in superscript).

1 revised El Escorial criteria a/b/c
2 defined as a score of 3 or lower on at least two of the three bulbar items of the ALSFRS-R (speech, salivorrhoea, swallowing) at follow-up
3 finger and foot tapping = (L+R)/2
Findings at follow-up are listed in Table 2. None of the PMA patients developed upper motor neuron signs or symptoms during the time of follow-up. When comparing the patient groups over time, vital capacity decreased significantly in ALS-B ($p = 0.013$) and PMA ($p = 0.028$), whereas the ALSFRS-R scores decreased significantly in ALS-B ($p = 0.011$), ALS-L ($p = 0.026$) and PMA ($p = 0.008$). Finger tapping speed was significantly lower at follow-up in ALS-B ($p = 0.038$) and ALS-L ($p = 0.018$). None of the clinical characteristics changed significantly over time in the controls.

**Figure 1.** Sagittal view of a reconstructed corticospinal tract, overlaid on the non-diffusion-weighted image.

**Diffusion tensor tractography: mean fractional anisotropy in the corticospinal tract**

The pilot study in which diffusion tensor tractography reproducibility was investigated yielded an inter-observer agreement of $88 \pm 8\%$. In $< 5\%$ of the cases, diffusion tensor tractography terminated in the pons. An example of a corticospinal tract reconstruction is shown in Figure 1 and mean fractional anisotropy values of the corticospinal tract at baseline and follow-up are listed in Tables 1 and 2, respectively. Representations of the reconstructed corticospinal tract in the sagittal and coronal plane per cohort are shown in Figure 2.

We found no significant differences of mean fractional anisotropy in the corticospinal tract between the hemisphere corresponding to the clinical side of onset and the
contralateral hemisphere. For all 23 samples along the corticospinal tract, corresponding fractional anisotropy values never differed > 1.5 % between hemispheres. Therefore, in each patient, we pooled fractional anisotropy values of both hemispheres for further analyses.

At baseline, a lower mean fractional anisotropy was found in PLS (\(p = 0.001\)) and ALS-B (\(p = 0.007\)) when compared to controls. In a direct comparison between patient groups, mean fractional anisotropy of the corticospinal tract in PMA was significantly higher than in PLS (\(p < 0.001\)) and ALS-B (\(p = 0.004\)).

At follow-up, mean fractional anisotropy of the corticospinal tract in ALS-L was significantly lower (\(p = 0.018\)) when compared to baseline.
Diffusion tensor tractography: fractional anisotropy profiles along the corticospinal tract

**Patient groups versus controls at baseline**

At baseline, fractional anisotropy in PLS was significantly lower compared with controls in the medulla oblongata and caudal pons, in the cerebral peduncle and posterior limb of the internal capsule, as well as in the subcortical white matter (Figure 3A).

**Figure 3.** Fractional anisotropy values along the caudocranial course of the corticospinal tract of patients (dotted line) with PLS (A), bulbar-onset ALS (B), limb-onset ALS (C) or PMA (D) compared to controls (bold line), and patients with (dotted line) bulbar symptoms compared to patients without (bold line) bulbar symptoms (E) at baseline. Mean fractional anisotropy values are shown ± the standard error of the mean in patients and controls in light and dark grey, respectively. Asterisks (*) and hash signs (#) indicate locations in which statistically significant differences between patient group and controls were found (Mann-Whitney U test, $p < 0.01$ in case of *, $p < 0.05$ in case of #). The middle image provides an anatomic reference.

**Abbreviations:** MO, medulla oblongata; P, pons; CP, cerebral peduncle; PLIC, posterior limb of the internal capsule; CR, corona radiata; SWM, subcortical white matter; ALS, amyotrophic lateral sclerosis; PLS, primary lateral sclerosis; PMA, progressive muscular atrophy
In ALS-B, fractional anisotropy was significantly lower compared with controls in the medulla oblongata, caudal pons, cerebral peduncle and posterior limb of the internal capsule (Figure 3B).

In ALS-L, fractional anisotropy was significantly lower compared with controls in the medulla oblongata and in the subcortical white matter (Figure 3C).

In PMA, fractional anisotropy was significantly higher compared with controls in the rostral part of the posterior limb of the internal capsule and the corona radiata, and lower in the subcortical white matter (Figure 3D).

Comparisons between patient groups at baseline
In PLS, fractional anisotropy was significantly lower in the distal medulla oblongata when compared with ALS-L, whereas FA was higher in the rostral part of the posterior limb of the internal capsule, and lower in the subcortical white matter when compared with ALS-B. Furthermore, fractional anisotropy was lower in the brainstem, posterior limb of the internal capsule and corona radiata of patients with PLS when compared with patients with PMA.

In ALS-B, fractional anisotropy was significantly lower in the medulla oblongata, pons, posterior limb of the internal capsule and corona radiata when compared with ALS-L. Furthermore, fractional anisotropy was lower in the brainstem, posterior limb of the internal capsule and corona radiata of patients with PLS when compared with patients with PMA.

Findings at follow-up
In ALS-B, fractional anisotropy decreased significantly over time in the cerebral peduncle/caudal part of the posterior limb of the internal capsule and in the subcortical white matter (Figure 4A).

In ALS-L, fractional anisotropy decreased significantly over time in the medulla oblongata, cerebral peduncle/caudal part of the posterior limb of the internal capsule and in the subcortical white matter (Figure 4B).

In PMA, fractional anisotropy increased significantly over time in the subcortical white matter (Figure 4C).

No significant fractional anisotropy changes were found in the controls over time (Figure 4D).

Voxel-based analysis: whole-brain white matter assessment
Patient groups versus controls at baseline
Significant fractional anisotropy reductions in patients with PLS compared with healthy controls were found in the medulla oblongata, caudal pons, cerebral peduncle, and posterior limb of the internal capsule. In ALS-L, fractional anisotropy was significantly lower compared with controls in the medulla oblongata and in the subcortical white matter. In PMA, fractional anisotropy was significantly higher compared with controls in the rostral part of the posterior limb of the internal capsule and the corona radiata, and lower in the subcortical white matter. Comparisons between patient groups at baseline revealed significant differences in the distribution of fractional anisotropy across different regions. Findings at follow-up showed significant changes over time in fractional anisotropy across different groups, with ALS-B showing significant decreases in the cerebral peduncle/caudal part of the posterior limb of the internal capsule and in the subcortical white matter, while ALS-L showed significant decreases in the medulla oblongata, cerebral peduncle/caudal part of the posterior limb of the internal capsule and in the subcortical white matter. PMA showed significant increases in fractional anisotropy in the subcortical white matter. No significant changes were observed in the controls.
controls at baseline were mainly located in the corticospinal tract, with the fractional anisotropy reduction extending from the subcortical white matter underneath the primary motor cortex to the cerebral peduncle, and in the body and splenium of the corpus callosum. Furthermore, reduced fractional anisotropy was found in the white matter underneath the primary sensory cortex, the right thalamus, the body of the fornix and the genu of the internal capsule (Figure 5A; Table 3).
Figure 5. Locations in which fractional anisotropy is significantly reduced in patients with PLS (A), bulbar-onset ALS (B), limb-onset ALS (C) or PMA (D) when compared to controls at baseline (non-parametric two-sample t-test, \( p < 0.001 \) uncorrected) are shown in red, overlaid on axial slices of the fractional anisotropy map of the population-based diffusion tensor imaging atlas. Specific anatomical locations/white matter structures are indicated by coloured arrows (see colour legend in figure).

Abbreviations: L, left; R, right; CST, corticospinal tract; CC, corpus callosum; IC, internal capsule; ALS, amyotrophic lateral sclerosis; PLS, primary lateral sclerosis; PMA, progressive muscular atrophy.
At baseline, a significant fractional anisotropy decrease in patients with ALS-B compared with controls was found mainly in the brainstem and the posterior limb of the internal capsule, and in the white matter underneath the left lateral precentral gyrus and the primary motor cortex, as well as in the genu of the internal capsule (Figure 5B, Table 3).

When comparing patients with ALS-L with controls at baseline, fractional anisotropy was significantly reduced in the body of the fornix, the right thalamus and the white matter underneath the primary motor cortex (Figure 5C, Table 3).

<table>
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<tr>
<th>Locations of fractional anisotropy reduction in patients when compared to controls at baseline, and when comparing patient data pair-wise over time.</th>
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<td>Abbreviations: PLS, primary lateral sclerosis; ALS-B, bulbar onset ALS; ALS-L, limb onset ALS; PMA, progressive muscular atrophy; L/R, difference present in left/right hemisphere; B, different present in both hemispheres; +, difference present in a midline structure; CST, corticospinal tract; WM, white matter; CC, corpus callosum</td>
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At baseline, a significant fractional anisotropy reduction in patients with PMA compared with controls was demonstrated in the white matter underneath the right primary motor cortex, the body of the fornix and the genu of the internal capsule (Figure 5D, Table 3).
Figure 6. Results of the direct comparisons between motor neuron disease phenotypes using voxel-based analysis, overlaid on axial slices of the fractional anisotropy map of the population-based diffusion tensor imaging atlas. In parts A-C, locations in which fractional anisotropy was significantly lower/higher in PLS compared to bulbar onset ALS (A), limb-onset ALS (B) or PMA (C) are shown in blue/yellow, respectively. In parts D and E, locations in which fractional anisotropy was significantly lower/higher in ALS-B compared to ALS-L (D) or PMA (E) are shown in yellow/blue, respectively. In part F, locations in which fractional anisotropy was significantly lower in ALS-L compared to PMA are shown in yellow. Specific anatomical locations/white matter structures are indicated by coloured arrows. Abbreviations: L, left; R, right; CST, corticospinal tract; CC, corpus callosum; IC, internal capsule; ALS, amyotrophic lateral sclerosis; PLS, primary lateral sclerosis; PMA, progressive muscular atrophy.
Comparisons between patient groups at baseline
Fractional anisotropy was significantly lower in PLS compared with ALS-B at baseline in the body and splenium of the corpus callosum and significantly higher in the posterior limb of the internal capsule, corona radiata, external capsule, thalamus and in the white matter of the inferior and superior temporal gyrus (Figure 6A, Table 4).
When comparing PLS with ALS-L at baseline, a significant fractional anisotropy reduction in PLS was found in the body of the corpus callosum and cerebral peduncle, whereas significantly higher fractional anisotropy in PLS was observed in the white matter underneath the dorsolateral prefrontal cortex, inferior temporal gyrus and the superior parietal lobule (Figure 6B, Table 4).

Table 4: Results of the voxel-based direct comparisons between MND phenotypes

<table>
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<tr>
<th></th>
<th>PLS vs ALS-B</th>
<th>ALS-B vs ALS-L</th>
<th>ALS-B vs PMA</th>
<th>ALS-L vs PMA</th>
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<td>CST</td>
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<td>cerebral peduncle</td>
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<td>posterior limb internal capsule</td>
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<td>corona radiata</td>
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<tr>
<td>corona radiata WM underneath primary motor cortex</td>
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<td>splenium</td>
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<td>WM underneath primary sensory cortex</td>
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<tr>
<td>WM of inferior frontal gyrus</td>
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<tr>
<td>WM of dorsolateral prefrontal cortex</td>
<td>↑</td>
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<td>WM of anterior temporal pole</td>
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<td>superior frontal</td>
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Locations of fractional anisotropy reductions; direct comparisons between the phenotypes at baseline.
Abbreviations: FA, fractional anisotropy; CST, corticospinal tract; CC, corpus callosum; WM, white matter; MND, motor neuron disease; PLS, primary lateral sclerosis; ALS-B, bulbar onset ALS; ALS-L, limb onset ALS; PMA, progressive muscular atrophy; vs, versus.
In a direct comparison of patients with PLS and PMA at baseline, fractional anisotropy was lower in PLS in the body and splenium of the corpus callosum and in the cerebral peduncle, posterior limb of the internal capsule and white matter underneath the primary motor cortex. Conversely, fractional anisotropy was higher in PLS in the anterior limb of the internal capsule (Figure 6C, Table 4).

Fractional anisotropy was significantly lower in ALS-B compared with ALS-L at baseline in the cerebral peduncle, posterior limb of the internal capsule and corona radiata, whereas the opposite was observed in the white matter underneath inferior frontal gyrus, superior frontal gyrus, superior parietal lobule and in the orbitofrontal white matter (Figure 6D, Table 4). The direct comparison between ALS-B and PMA at baseline yielded significantly lower fractional anisotropy in the patients with ALS-B throughout the corticospinal tract and in the white matter of the anterior temporal pole and superior temporal gyrus and significantly higher fractional anisotropy in the external capsule, the cingulum and the orbitofrontal white matter of the patients with ALS-B (Figure 6E, Table 4).

When comparing ALS-L directly to PMA at baseline, significantly lower fractional anisotropy values were observed in the patients with ALS-L in the posterior limb of the internal capsule, corona radiata and white matter underneath the primary motor and sensory cortex and superior parietal lobule. No significantly higher fractional anisotropy values were found in ALS-L compared to PMA (Figure 6F, Table 4).

Findings at follow-up
When comparing the baseline and follow-up scans of 9 patients with ALS-B, an extensive pattern of fractional anisotropy reduction over time was found comprising the white matter underneath the primary motor and sensory cortex, premotor cortex, inferior frontal gyrus and dorsolateral prefrontal cortex, as well as the body and genu of the corpus callosum, the left thalamus, the hippocampal formations and the right cingulum (Figure 7A, Table 3).

When comparing the baseline and follow-up scans of 7 patients with ALS-L, fractional anisotropy reduction over time was found throughout the corticospinal tract, in the body of the corpus callosum, in the white matter underneath primary sensory cortex and anterior temporal pole, the right thalamus and cingulum, as well as the left optic radiations (Figure 7B, Table 3).
Figure 7. Locations in which fractional anisotropy is significantly reduced in patients with bulbar-onset ALS (A), limb-onset ALS (B) or PMA (C) over time (non-parametric paired t-test, $p < 0.001$ uncorrected) are shown in green, overlaid on axial slices of the fractional anisotropy map of the population-based diffusion tensor imaging atlas. Specific anatomical locations/white matter structures are indicated by coloured arrows (see colour legend in figure).

Abbreviations: L, left; R, right; CST, corticospinal tract; CC, corpus callosum; ALS, amyotrophic lateral sclerosis; PLS, primary lateral sclerosis; PMA, progressive muscular atrophy
When comparing fractional anisotropy over time in 10 patients with PMA, a pattern of fractional anisotropy reduction over time was found in the white matter underneath the primary motor and sensory cortex, premotor cortex, inferior frontal gyrus, dorsolateral prefrontal cortex and the lateral precentral gyrus, as well as in the genu and body of the corpus callosum, the cingulum, the left hippocampal formations and the body of the fornix (Figure 7C, Table 3).

No significant fractional anisotropy changes over time were found in the control group.

DISCUSSION

Spatial profiling along the corticospinal tract showed fractional anisotropy changes in all motor neuron disease phenotypes. The corticospinal tract is a white matter tract that shows a distinct morphology. Anatomically, the tract fibres are very coherent and tightly packed in the internal capsule and more so in the cerebral peduncle, whereas the fibers tend to fan out at the more cranial levels of the corticospinal tract. These varying degrees of fibre coherence and fibre packing at different levels of the corticospinal tract will be reflected by fractional anisotropy values varying widely over the caudocranial course of the corticospinal tract, as was also demonstrated in all cohorts in our spatial profiling study, with additional differences in fractional anisotropy between patients and controls that may reflect the in vivo pathology. In general, fractional anisotropy reductions were modest in PMA and most extensive in PLSD. For the corticospinal tract, the results of the diffusion tensor tractography and voxel-based analysis study were largely overlapping. The voxel-based analysis study demonstrated varying extents of white matter involvement in the different phenotypes, albeit in quite similar anatomical locations.

Over time, more extensive patterns of fractional anisotropy reduction were observed with voxel-based analysis than with diffusion tensor tractography. In the following paragraphs, we provide a comparison of our findings to previous diffusion tensor imaging studies and attempt to relate our findings to clinical observations in each phenotype and models of pathogenesis in ALS.

Previous diffusion tensor imaging studies

First, it should be noted that a direct comparison of different studies applying diffusion tensor tractography or voxel-based analysis in motor neuron disease phenotypes is not trivial. A factor hindering objective comparison is that in most previous studies, varying
proportions of patients with ALS-L, ALS-B, PLS or PMA were included. It is unclear to which extent the results of these studies were influenced by the presence of either phenotype in the patient populations. Second, the patient populations also differed in disease severity and duration, further complicating an objective comparison. Finally, the results of diffusion tensor imaging studies may depend on the data processing approach that is adopted for the study, which has already been demonstrated previously for voxel-based analysis approaches. In this study, the results of the diffusion tensor tractography- and voxel-based analysis study yielded largely overlapping, but sometimes also conflicting results. All these factors underline the need for standardization of inclusion criteria, data acquisition and processing in future investigations.

General comments
The strength of this study is that we carefully defined subtypes of motor neuron disease at an early stage of disease and included equal numbers per group with a systematic follow-up after 6 months. Unfortunately, we still lost almost a third of our patients to follow-up because of rapid disease progression. This may be related to the fact that our inclusion criteria introduced a selection bias towards patients with a rapidly progressive disease course. It is well known that a shorter delay until diagnosis is associated with a more aggressive disease course. Delay until diagnosis in our study was 6.6 months on average, while the diagnostic delay in epidemiological surveys is 12 months on average. A limitation of our fibre tracking study is that we calculated multiple p-values which increased the probability of a type-I error. We decided to not perform a multiple testing correction method for two reasons. First, the sample size of this study may not provide adequate power to perform such a correction. Second, since the correction methods assume independence between the multiple p-values their application to the highly dependent fractional anisotropy values along the corticospinal tract would mean that the corrected p-values would be too conservative. The same holds true for the voxel-based analysis study, for which we adopted a threshold of p < 0.001 uncorrected to avoid underestimation of effects in our relatively small cohorts. The same statistical threshold was adopted in a previous voxel-based analysis study in patients with ALS, in which the threshold was chosen according to previous reports and to a priori hypothesis based on available studies. We found no significant differences of mean fractional anisotropy in the corticospinal tract between the hemisphere corresponding to the clinical side of onset and the contralateral hemisphere, which is in line with previous diffusion tensor imaging studies of the corticospinal tract. This may be explained by the fact
that the clinical side of onset is also and sometimes predominantly determined by lower motor neuron involvement.

**Primary lateral sclerosis**

*Corticospinal tract*

We demonstrated lower fractional anisotropy along the entire course of the corticospinal tract in patients with PLS patients when compared to controls in both the diffusion tensor imaging - and voxel-based analysis study. Previously, a fractional anisotropy reduction within the posterior limb of the internal capsule of patients with PLS (mean disease duration 5.1 years) was reported when compared to controls in a region-of-interest analysis. A recent voxel-based analysis reported more widespread fractional anisotropy reduction in a cohort of 25 patients with PLS, which resembled our observations.

In direct comparisons between the motor neuron disease phenotypes, fractional anisotropy was generally lower in the corticospinal tract of PLS compared with other groups. Similarly, lower fractional anisotropy values in the rostral section of the corticospinal tract of 6 patients with PLS were reported (mean disease duration 7.4 years) when compared with patients with ALS. This suggests more advanced corticospinal tract degeneration in PLS than in other patient groups, which may be explained by the upper motor neuron nature of PLS in combination with a longer disease duration. Differences with PMA were most marked, with a lower fractional anisotropy in PLS along a large part of the corticospinal tract in both the diffusion tensor tractography and voxel-based analysis. This may be understood as PLS is clinically at the upper motor neuron end of the motor neuron disease spectrum and PMA at the lower motor neuron end. When comparing PLS and ALS-B to controls, fractional anisotropy was decreased in the posterior limb of the internal capsule in both patient groups, but in a mutual comparison between PLS and ALS-B this decrease seemed to extend slightly more rostrally in ALS-B than in PLS.

*Extra-motor findings*

An extensive pattern of extra-motor fractional anisotropy reduction was demonstrated in PLS when compared with controls in the voxel-based analysis study, including the body and splenium of the corpus callosum, the white matter underneath the primary sensory cortex, thalamus, the body of the fornix and the genu of the internal capsule. Two other studies reported reduced fractional anisotropy in the white matter underneath the primary motor cortex and in the body of the corpus callosum compared with controls.
which is very similar to our findings. Likewise, reduction of white matter volume and \(^{15}\text{C}\)-flumazenil binding were recently reported in similar areas of the motor cortex and corpus callosum. Also when compared to other motor neuron disease phenotypes, fractional anisotropy was significantly lower within the body and splenium of the corpus callosum in PLS. Reduced fractional anisotropy in the body of the corpus callosum in patients with PLS may reflect the pronounced degeneration of collaterals of the CST that connect the motor cortices that was described earlier. The interpretation of the observed fractional anisotropy reduction in the splenium of the corpus callosum may be difficult, as the splenium does not carry homogeneous fibers connecting single functional cortical areas. However, as the temporal cortex axons pass through the ventral isthmus or ventral splenium, parietal cortex axons connect via the dorsal part of the splenium, while axons from the visual cortices pass through the ventral pole of the splenium, our observations of reduced fractional anisotropy in the splenium of the corpus callosum in patients with PLS may correspond to previous findings of parietal involvement in patients with PLS.

The demonstration of reduced fractional anisotropy within the genu of the internal capsule of patients with PLS when compared with controls is an interesting finding, since the motor neurons innervating the head, neck and tongue originate in the lateral precentral gyrus and descend through the genu of the internal capsule to the cranial nuclei in the brainstem, thus forming the corticobulbar tracts. Reduced fractional anisotropy within the genu of the internal capsule in PLS may therefore provide an imaging substrate of the clinical pseudobulbar signs, as pseudobulbar affect was observed in all save one patient (data not shown).

Fractional anisotropy reductions were demonstrated in the body of the fornix. The fornix is a major output white matter tract between the hippocampal formation and other limbic structures, thus being involved in emotional processing and memory. On detailed neuropsychological evaluation, mild cognitive impairment has been found in PLS, especially in frontal lobe function and memory. Unfortunately, no detailed neuropsychological assessment of any of our patient groups was performed, so we cannot directly validate this in our population.

Finally, we also observed a fractional anisotropy reduction in the thalamus of patients with PLS when compared with controls. In previous diffusion tensor imaging studies, distinct subregions were identified in the thalamus, whose locations corresponded to
nuclei described previously in histological studies and which could be related to functional anatomical divisions within the thalamus in a probabilistic atlas. The fractional anisotropy reduction in the thalamus, which we observed in our cohort of patients with PLS when compared with controls, was located in the ventral anterior nucleus, which was assigned to the part of the thalamus projecting to the prefrontal cortices. Since the prefrontal cortices are mainly involved in cognitive processes, this observation may again be related to the previously mentioned possible cognitive impairment in patients with PLS.

**Bulbar- and limb-onset amyotrophic lateral sclerosis**

**Corticospinal tract**

When comparing ALS-L and ALS-B to controls at baseline, the diffusion tensor imaging and voxel-based analysis study yielded similar results, with the fractional anisotropy reduction in ALS-L being limited to the rostral parts of the corticospinal tract, and fractional anisotropy decline over time throughout the entire corticospinal tract. Conversely, the caudal parts of the corticospinal tract in ALS-B were most severely affected early in the disease, whereas the more rostral parts became involved at a later stage.

However, our study also yielded conflicting results, with no significant differences between ALS-L and ALS-B in the diffusion tensor tractography study, but significantly lower fractional anisotropy in the caudal parts of the corticospinal tract in ALS-B in the voxel-based analysis study when comparing these patient groups directly. Differing extent of upper motor neuron involvement between ALS-L and ALS-B in our study cannot be explained by differing disease severity or duration between the groups, as these were similar at either time point.

Only few previous diffusion tensor imaging studies assessed patients with ALS-L and ALS-B as separate groups, as in most studies patients with ALS-L and ALS-B were pooled to obtain a single study population, or no information was provided on the onset type. Moreover, most of these studies focused on (parts of) the corticospinal tract by either performing region-of-interest analyses or fibre tracking. Lower fractional anisotropy in the corticospinal tract of patients with ALS-B compared with controls, and lower mean fractional anisotropy in ALS-B compared with ALS-L were observed in previous studies, although the latter was not confirmed.
A limited number of studies performed spatial profiling along the corticospinal tract, none of which included separate groups of patients with ALS-L and ALS-B. In previous diffusion tensor tractography studies, significantly lower fractional anisotropy values in the posterior limb of the internal capsule and in the subcortical white matter of the corticospinal tract in a mixed group of 28 ALS patients with a disease duration of 4–34 months, and decreased fractional anisotropy values in the cerebral peduncle in a spatial profiling diffusion tensor imaging study of the part of the corticospinal tract between cerebral peduncle and the corona radiata in 14 patients with ALS with a disease duration of $22 \pm 12$ months were found. $^{17, 54}$ Similar observations were reported in a diffusion tensor tractography study in a mixed group of 46 patients with ALS with a disease duration of 6–49 months. $^{27}$ Other studies comparing sporadic patients with ALS with controls using voxel-based analysis or tract-based spatial statistics reported more extensive patterns of fractional anisotropy reduction within the corticospinal tract of patients with ALS at a more advanced stage of the disease. $^{18, 26, 29, 32, 72}$

There are only few longitudinal diffusion tensor imaging studies providing data on fractional anisotropy changes in ALS, all of which failed to demonstrate a decrease of mean fractional anisotropy of the corticospinal tract over time. $^{17, 33, 34}$ However, in the voxel-based analysis study, significant fractional anisotropy reductions over time were observed throughout the CST in both ALS-L and ALS-B, suggesting that our optimized voxel-based analysis approach might be more sensitive to assess the diffusion tensor imaging data longitudinally.

**Extra-motor findings**

Other voxel-based analysis or tract-based spatial statistics studies comparing sporadic patients with ALS-L with controls not only found more extensive patterns of fractional anisotropy reductions in such patients within the corticospinal tract, but also in the corpus callosum, the frontal white matter and thalami. $^{17, 18, 26, 29, 32, 72}$ The disease duration of the patients with ALS-L in these studies was generally longer than that of our patients with ALS-L ($10.5 \pm 2.5$ months), which might explain the more extensive patterns of fractional anisotropy reduction.

The reduced fractional anisotropy in the body of the corpus callosum over time in patients with ALS-L and ALS-B in this study and compared with controls in previous studies $^{18, 26, 29, 32}$ might relate to the observation of impaired transcallosal inhibition and mirror movements in a number of patients with ALS $^{74, 75}$, as anatomically, the central part
of the corpus callosum contains inter-hemispheric fibers between the motor cortices. The reduced fractional anisotropy over time in the genu of the corpus callosum in ALS-B may be related to the cognitive impairment that is often reported in patients with ALS 15, 16, since the genu of the corpus callosum connects orbitofrontal and (pre)frontal cortices that subserve cognitive processes. Previously, it was demonstrated that severe atrophy in the anterior half of the corpus callosum is associated with cognitive decline and psychiatric symptoms in ALS 75, although it is unclear whether this study population also included patients with ALS-B. It has been suggested that cognitive impairment may be more prevalent in patients with ALS-B than in patients with ALS-L 15, 77, 78, although this finding was not confirmed in another study 16. In our study, different structures involved in cognition and memory were impaired in patients with ALS-B, whereas in patients with ALS-L, the fractional anisotropy reduction was mainly limited to structures that are mostly involved in motor tasks. This may explain why the genu of the corpus callosum was more involved in our patients with ALS-B than in those with ALS-L. However, we did not perform neuropsychological testing to prove this hypothesis.

Additionally, fractional anisotropy was decreased in the white matter of the inferior frontal gyrus and dorsolateral prefrontal cortex of the patients with ALS-B when compared with controls, two frontal areas involved in executive function 79 and working memory 80. The inferior frontal gyrus plays an important role in verbal fluency, which is most frequently reported impaired in patients with ALS 13, 15, 16. A recent meta-analysis showed a relation between visual and verbal memory impairments in patients with ALS and frontal lobe involvement. 16 In this view, the fractional anisotropy reductions in the hippocampal formations and cingulum over time in the patients with ALS-B and in the frontal white matter in patients with ALS-L when compared with other phenotypes, may reflect such functional impairments. Similar reductions in the frontal white matter were reported in other voxel-based analysis- or tract-based spatial statistics studies, when comparing patients with sporadic ALS-L with controls. 17, 18, 29

When comparing ALS-B directly with other motor neuron disease phenotypes, the most striking observation was that fractional anisotropy in the temporal white matter of the patients with ALS-B was lower than in any of the other phenotypes. The white matter underneath the inferior temporal gyrus was also affected in ALS-L when compared directly to PLS. As the temporal lobe is highly involved in auditory, language and also memory processing, abnormalities of the temporal lobe may further contribute to the previously described decline in cognitive functioning in ALS 15, 16.
Fractional anisotropy was reduced within the genu of the internal capsule and the lateral precentral gyrus of patients with ALS-B when compared with controls. Similar to the findings in the patients with PLS, reduced fractional anisotropy within the genu of the internal capsule and white matter of the lateral precentral gyrus in ALS-B may provide evidence for impairment of the corticobulbar tracts in ALS-B, as pseudobulbar affect was observed in 7 and 9 patients at baseline and at follow-up, respectively.

Finally, in both patients with ALS-B and with ALS-L, we demonstrated reduced fractional anisotropy in the thalamus when compared with controls. In patients with ALS-B, this fractional anisotropy was located in the part of the ventral anterior nucleus projecting to the prefrontal cortices as described in a previous study, which is similar to the location in which we found a fractional anisotropy reduction in patients with PLS compared with controls. Conversely, in the patients with ALS-L, the fractional anisotropy reduction in the thalamus appeared to be located in the ventral anterior nucleus, which was assigned to the part of the thalamus projecting to the primary motor and premotor cortices. Together with the fractional anisotropy changes in ALS in the white matter underneath the primary somatosensory cortex, premotor cortex and superior parietal lobe, this may represent secondary degeneration of tracts involved in voluntary motor control.

Progressive muscular atrophy

Corticospinal tract

For the corticospinal tract, the results of the diffusion tensor tractography- and voxel-based analysis study were not completely overlapping. In both studies, a significantly lower fractional anisotropy was found in the white matter underneath the primary motor cortex in patients with PMA when compared with controls. However, spatial profiling in our study revealed a higher FA compared with controls in the rostral internal capsule/corona radiata, which was not observed in the voxel-based analysis study. Anatomically, at the level of the corona radiata, the corticospinal tract crosses with fibre tracts of the corpus callosum and the superior longitudinal fasciculus, leading to low fractional anisotropy values. A selective loss of corticospinal tract fibres at that level may turn the corona radiata into a seemingly more organized structure, reflected in higher fractional anisotropy values. Therefore, one explanation might be that increased fractional anisotropy in a region with intersecting fibers is indicative of corticospinal tract degeneration, as was described previously in ALS and other neurodegenerative diseases, although it remains purely hypothetical whether this explanation holds true in our PMA cohort. Another explanation may be the inherent heterogeneity of the
relatively small groups included in this study. Thus, the clinical relevance of this finding may be limited.

For the direct comparisons between motor neuron disease phenotypes, the results show more overlap. When comparing PMA with ALS in the diffusion tensor tractography- and voxel-based analysis study, fractional anisotropy was significantly lower in the caudal, middle and rostral section of the corticospinal tract in ALS-L, and in the caudal and middle section of the corticospinal tract in ALS-B. These findings suggest that, compared with controls, there is evidence of corticospinal tract involvement in PMA, but corticospinal tract involvement in both ALS groups seems more severe.

Previous diffusion tensor imaging studies in PMA were based on region-of-interest analysis and yielded contradicting findings. Two studies found no differences between PMA and controls \(30, 34\), whereas another study demonstrated decreased fractional anisotropy in the rostral section of the corticospinal tract in patients with a lower motor neuron phenotype \(36\). However, all patients of the latter study developed upper motor neuron signs in a later course of their disease, whereas none of our patients had clinical evidence of upper motor neuron involvement at any time point. Another study showed fractional anisotropy values similar to patients with ALS in a small subset of patients with PMA at an advanced stage of disease.\(^{31}\) Reduced fractional anisotropy in the corticospinal tract of patients with PMA may correspond to histological findings of corticospinal tract degeneration in patients without any evidence of upper motor neuron signs during life.\(^{5, 6, 84}\)

**Extra-motor findings**

The fractional anisotropy reduction in the genu of the internal capsule of patients with PMA may again represent a degeneration of the corticobulbar tracts, although only 2 of the 12 patients with PMA had bulbar symptoms at baseline. Likewise, the fractional anisotropy reduction in the white matter underneath the primary sensory and premotor cortex could represent secondary degeneration of the motor system.

Surprisingly, we found a reduction of fractional anisotropy in the white matter of the inferior frontot gyrus, dorsolateral prefrontal cortex, hippocampal formations and the body of the fornix of patients with PMA over time. An early study demonstrated impaired visual memory and attention in patients with PMA \(85\), which could not be confirmed in a more recent study \(86\). Another recent study found impaired category fluency and working
memory in these patients. Therefore, reduced fractional anisotropy within the structures for executive functioning and memory may provide anatomical substrates for cognitive dysfunction in patients with PMA. The observation of a fractional anisotropy reduction in the genu of the corpus callosum over time in the patients with PMA further provides support for cognitive involvement in PMA.

The spectrum of motor neuron disease and the El Escorial classification criteria
In this study, we demonstrated both upper motor neuron and extra-motor white matter involvement in all phenotypes of motor neuron disease, which was progressive in ALS-L, ALS-B and PMA. These observations illustrate one of the main advantages of diffusion tensor imaging. Using diffusion tensor tractography and voxel-based analysis, we were able to assess the anatomic distribution and even the spread of white matter involvement in the different motor neuron disease phenotypes in vivo, which has not been possible with other imaging or neurophysiological techniques. Our findings suggest that ALS, PLS and PMA are all part of a single spectrum of multisystem neurodegenerative disease currently described by the term ‘motor neuron disease’, in contrast to the view of ALS, PLS and PMA being separate entities, as is reflected in the current classification of patients with ALS. This classification is based on the revised El Escorial criteria, which are based on clinical evidence of upper motor neuron in combination with lower motor neuron involvement, and actually exclude patients with PLS or PMA.

Spread of pathology in motor neuron disease
Several hypotheses have been postulated regarding the disease pathogenesis in terms of disease onset and subsequent spread.

“Dying back” versus “dying forward” degeneration
In the 1990s it was postulated that ALS is primarily a disease of the upper motor neuron, which recruits the lower motor neuron into the degenerative process through an anterograde ‘dying forward’ process. In contrast, others proposed that the onset of neuronal degeneration in ALS is targeted at the lower motor neuron level, with spreading to the upper motor neuron by a retrograde or ‘dying-back’ process. The validity of either of these hypotheses has been debated widely.

Dual focality of disease onset
More recently, a clinical spatiotemporal analysis of disease progression in 100 patients with early ALS revealed that, although upper motor neuron and lower motor
neuron signs were both most conspicuous in the region of onset, further progress and extension to other body regions were essentially independent of each other, supporting a simultaneous and independent process of degeneration in upper and lower motor neurons. Since then, dual focality of onset (cortex and anterior horn) with contiguous spread outwards from these foci in both lower and upper motor neuron pathways is one of the most widely supported views of ALS pathogenesis.

Based on our study, it is difficult to comment on hypotheses regarding the level and/or focality of disease onset, or regarding the possible relationship between upper and lower motor neuron degeneration, as we only obtained diffusion tensor imaging data at the upper motor neuron level, and our patients with ALS-L and ALS-B had, by definition, clinical evidence of both upper and lower motor neuron degeneration at the time of scanning. The observed differences in the distribution and extent of upper motor neuron involvement between ALS-B and ALS-L do not seem substantial, nor do these provide convincing evidence of a ‘dying back’ or ‘dying forward’ degeneration of the corticospinal tract in ALS. For instance, in ALS-L, fractional anisotropy reductions were found at both ends of the corticospinal tract when compared with controls at baseline in the diffusion tensor tractography study, skipping the middle section, which became involved at follow-up, thus not supporting a unidirectional spreading phenomenon along the corticospinal tract. However, the patterns of spreading of pathology over time in ALS-L, ALS-B and PMA appeared rather similar, especially in ALS-L and PMA. These results are in line with the view of contiguous outward spread. Although further study is required with more subjects per motor neuron disease phenotype to validate our findings, our study shows that diffusion tensor imaging can be a valuable tool in evaluating the upper motor neuron and extra-motor involvement at early stages of the disease and the spread of pathology over time.
CONCLUSION

Upper motor and extra-motor neuron involvement were observed in all phenotypes of motor neuron disease shortly after diagnosis, suggesting that ALS, PLS and PMA are all part of the same spectrum of multisystem neurodegenerative disease. Voxel-based analysis was more sensitive to detect longitudinal changes than diffusion tensor tractography of the corticospinal tract. Voxel-based analysis may be particularly valuable in the evaluation of motor and extra-motor white matter involvement in the early symptomatic stages of motor neuron disease, and for monitoring the spread of pathology over time.
chapter 7

REFERENCES


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