Upper and extra-motoneuron involvement in early motoneuron disease: a diffusion tensor imaging study

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Motoneuron disease is a term encompassing three phenotypes defined largely by the balance of upper versus lower motoneuron 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. To further delineate the phenotypes within the motoneuron disease spectrum, this controlled study assessed the upper- and extra-motoneuron white matter involvement in cohorts of patients with motoneuron disease phenotypes shortly after diagnosis by comparing diffusion tensor imaging data of the different cohorts to those of healthy controls and directly between the motoneuron 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 comparison of the sensitivity of these techniques to detect white matter involvement in different phenotypes of motoneuron 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. The observation of both upper motor and extra-motoneuron involvement in all phenotypes of motoneuron 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 analyses may be particularly valuable in the evaluation of motor and extra-motor white matter involvement in the early symptomatic stages of motoneuron disease, and for monitoring the spread of pathology over time.

Keywords: amyotrophic lateral sclerosis; motoneuron disease; diffusion tensor imaging; fibre tracking; corticospinal tract

Abbreviations: ALS = amyotrophic lateral sclerosis; ALSFRS-R = revised amyotrophic lateral sclerosis functional rating scale; PLS = primary lateral sclerosis; PMA = progressive muscular atrophy

Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease, characterized by progressive upper and lower motoneuron signs. The primary target of disease remains unknown (Chou and Norris, 1993; Eisen, 1995; Ravits and La Spada, 2009). Progressive muscular atrophy (PMA) is defined by progressive lower motoneuron signs and diagnosed after exclusion of other lower motoneuron syndromes (Visser et al., 2002). Its prognosis resembles that of ALS and upper motoneuron signs may occur in the course of the disease (Brownell et al., 1970; Ince et al., 2003; Visser et al., 2007; Kim et al., 2009). Primary lateral sclerosis (PLS) is a pure upper motoneuron syndrome of slowly progressive, usually spino-bulbar, spasticity and is typically associated with a much longer survival (Pringle et al., 1992; Gordon et al., 2006).

The exact relationship between PMA, ALS and PLS remains unresolved. ALS may well encompass various phenotypes with different aetiologies or different modifying factors (Gordon et al., 2006; Rosenfeld and Swash, 2006; Wijesekera and Leigh, 2009). 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 (Abrahams et al., 1997; Lomen-Hoerth et al., 2002; Phukan et al., 2007; Sage et al., 2007, 2009; Raaphorst et al., 2010).

Diffusion tensor imaging is able to characterize the diffusion properties of water molecules in vivo (Basser et al., 1994). 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 (Basser and Pierpaoli, 1996). 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 (Beaulieu, 2002). In particular, it has been suggested that a reduction in anisotropy can reflect axonal fibre degeneration and myelin breakdown in both the peripheral and central nervous system (Beaulieu et al., 1996; Ciccarelli et al., 2006).

With diffusion tensor tractography it is possible to reconstruct specific major white matter tracts (Wakana et al., 2004) such as the corticospinal tract. 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 (Sach et al., 2004; Müller et al., 2009; Sage et al., 2009; Senda et al., 2009).

Previous diffusion tensor imaging studies found decreased fractional anisotropy to be related to clinical (Sage et al., 2009) and electrophysiological (Sach et al., 2004; Iwata et al., 2008) measures of upper motoneuron degeneration in patients with ALS, indicating that diffusion tensor imaging has potential in the objective evaluation of upper motoneuron degeneration in ALS. However, most studies only included typical patients with ALS, with few studies investigating different motoneuron disease phenotypes concurrently (Graham et al., 2004; Cosottini et al., 2005; Ciccarelli et al., 2009) or longitudinally (Agosta et al., 2007; Blain et al., 2007; Mitumoto et al., 2007; Sage et al., 2007). 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 motoneuron 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 motoneuron 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 motoneuron disease.

Materials and methods

Subjects

We included cohorts of patients with bulbar-onset ALS, limb-onset ALS, 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.vdG.).

All patients with ALS met the revised El Escorial criteria for probable, probable laboratory supported or definite ALS (Brooks et al., 2000).

PWA was diagnosed when clinical and electrophysiological evidence of progressive pure lower motoneuron involvement was present in two or more regions, excluding patients with focal or segmental muscular atrophy, and after ruling out other lower motoneuron diseases by extensive neurophysiological testing and neuroimaging if necessary (Brooks et al., 2000; Visser et al., 2007). 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 studied including patients with an apparently sporadic, adult-onset idiopathic upper motoneuron 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) (Gordon et al., 2006), and alternative diagnoses had been ruled out by extensive neurophysiological and genetic testing (Brugman et al., 2009). 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 patients. Follow-up visit for all cohorts, except PLS, was 6 months after baseline. Patients with PLS only provided baseline data, meant to obtain reference values for longstanding and prominent upper motoneuron 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/10 s, subsequently expressed as taps/s after averaging values of the left and right side), as deceleration of tapping speed suggests a upper motoneuron lesion (Kent-Braun et al., 1998). 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 (Cedarbaum et al., 1999). We used the score on the three bulbar items of the ALSFRS-R (speech, sialorrhoea and swallowing, a score of 4 per item representing normal function) to define the presence of bulbar involvement in all groups. We defined bulbar involvement as a score 3 or lower on at least two of the three bulbar items of the ALSFRS-R. Furthermore, we calculated the disease progression rate as follows:

\[
\text{Disease progression rate} = \frac{48 - \text{ALSFRS-R score}}{\text{disease duration}} \quad \text{(Ellis et al., 1999)}.
\]

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/mm\(^2\) (Akkerman, 2003). Additionally, one set of images was acquired without diffusion weighting (\(b = 0\) s/mm\(^2\)). Other sequence parameters were: 64 contiguous axial slices, echo time = 94 ms, repetition time = 8115 ms, field-of-view = 250 mm, scan matrix = 109 \times 112, interpolated image matrix = 256 \times 256, slice thickness = 2.2 mm, non-interpolated voxel size = 2.2 \times 2.2 \times 2.2 \text{mm}^3. Diffusion tensor imaging scan time was \sim 8\text{min.}

Data processing

The raw diffusion-weighted images were motion and eddy current corrected using a linear affine registration in the phase direction (Mangin et al., 2001) and the images were filtered for noise using the Linear Minimum Mean Square Estimator (Aja-Fernández et al., 2007). 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 (Landman et al., 2007; Leemans and Jones, 2009). The diffusion tensor was estimated from the corrected diffusion-weighted images and from this fractional anisotropy was derived (Basser and Pierpaoli, 1996).

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 (Wakana et al., 2004). 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 (Wakana et al., 2004; Sage et al., 2007).

Diffusion tensor tractography is known to be a user-dependent process (Wang and Melhem, 2005). Therefore, fibre tracking reproducibility was assessed by two blinded observers (M.M.vdG. and 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.vdG.) 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 (Mori et al., 2008) using joint affine and non-rigid registration using smooth basis functions (Ashburner and Friston, 1999). 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 resided in one common reference frame. To increase the signal-to-noise ratio and reduce the number of data points these data sets were then resampled at a resolution of 4 mm, yielding fractional anisotropy profiles sampled at 23 positions in a
structures while removing noise in homogeneous regions. As recommended in a recent paper (Van Hecke et al., 2010), anisotropy maps were anisotropically smoothed (Sijbers et al., 1999). Finally, the warped fractional anisotropy maps were anisotropically smoothed (Sijbers et al., 1999), as recommended in a recent paper (Van Hecke et al., 2010). This type of smoothing preserves the boundaries and sharpens edges between structures while removing noise in homogeneous regions.

**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. (2009), 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 to those obtained by tract-based spatial statistics (Smith et al., 2006). In short, data processing included coregistering all diffusion tensor imaging data sets non-rigidly (Van Hecke et al., 2006) to a population-based diffusion tensor imaging atlas, which was generated from the diffusion tensor imaging data from all subjects included in this study (Van Hecke et al., 2008). Finally, the warped fractional anisotropy maps were anisotropically smoothed (Sijbers et al., 1999), as recommended in a recent paper (Van Hecke et al., 2010). 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 patients 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 analysed with the Wilcoxon signed-rank 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 Parametric Mapping toolbox available for SPM5 (Statistical Parametric Mapping, University College London) (Nichols and Holmes, 2002). 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 (limb-onset ALS/bulbar-onset ALS/PLS/PMA at baseline versus controls at baseline).

Furthermore, we also performed direct comparisons between the different motoneuron 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.

**Results**

At follow-up, 7 out of 12 limb-onset ALS, 9 out of 12 bulbar-onset ALS, 10 out of 12 patients with PMA and 12 controls were able to undergo a repeated scan session. Reasons for lost-to-follow-up included (i) death (three limb-onset ALS and two patients with PMA); (ii) too disabled to visit the hospital (one limb-onset ALS and two bulbar-onset patients with ALS) and (iii) inability to lie in supine position (one limb-onset ALS and one patient with bulbar-onset ALS). The baseline and follow-up data of one control subject had to be discarded due to imaging artefacts.

**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 12 patients with PLS had a disease duration of <3 years (19–28 months) upon inclusion. These patients were re-examined 1.5 years after the baseline scan. None of them had developed lower motoneuron 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 motoneuron syndrome. When comparing the different patient groups, disease progression rate at baseline was significantly slower in PLS than in limb-onset ALS ($P = 0.003$), bulbar-onset ALS ($P = 0.013$) and PMA ($P = 0.009$). Vital capacity at baseline did not differ significantly between any of the patient groups, but was significantly lower in bulbar-onset ALS ($P = 0.043$) and PLS ($P = 0.028$) when compared with controls. The ALSFRS-R score was significantly lower in PLS than in limb-onset ALS ($P = 0.010$), bulbar-onset ALS ($P = 0.009$) and PMA ($P = 0.001$) and in all motoneuron disease phenotypes when compared with controls ($P < 0.001$ for all comparisons). Finger tapping speed was significantly lower in PLS than in bulbar-onset ALS ($P = 0.004$), PMA ($P < 0.001$) and controls ($P < 0.001$), significantly lower in limb-onset ALS than in PMA ($P = 0.006$) and controls ($P = 0.002$), and significantly lower in bulbar-onset ALS than in controls ($P = 0.013$). Disease progression rate, vital capacity and ALSFRS-R score did not differ significantly
between PMA and limb-onset ALS or bulbar-onset ALS, or between limb-onset ALS and bulbar-onset ALS.

Findings at follow-up are listed in Table 2. None of the patients with PMA developed upper motoneuron signs or symptoms during the time of follow-up. When comparing the patient groups over time, vital capacity decreased significantly in bulbar-onset ALS (P = 0.013) and PMA (P = 0.028), whereas the ALSFRS-R scores decreased significantly in bulbar-onset ALS (P = 0.011), limb-onset ALS (P = 0.026) and PMA (P = 0.008). Finger tapping speed was significantly lower at follow-up in bulbar-onset ALS (P = 0.018) and limb-onset ALS (P = 0.018). None of the clinical characteristics changed significantly over time in the controls.

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 ± 8%. In < 5% of the cases, diffusion tensor tractography terminated in the pons. An example of a corticospinal tract reconstruction is shown in Fig. 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 Supplementary Fig. 1.

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 bulbar-onset ALS (P = 0.007) when compared with 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 bulbar-onset ALS (P = 0.004).

### Table 1 Baseline data of four cohorts of motoneuron disease phenotypes and healthy controls

<table>
<thead>
<tr>
<th></th>
<th>PLS (n = 12)</th>
<th>Bulbar-onset ALS (n = 12)</th>
<th>Limb-onset ALS (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>No of patients with bulbar symptoms</td>
<td>8</td>
<td>12</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Revised El Escorial classification a/b/c</td>
<td>NA</td>
<td>2/B/2</td>
<td>1/0/1</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Age, years (median/range)</td>
<td>59.0 (48–74)</td>
<td>56.5 (42–72)</td>
<td>58.0 (41–78)</td>
<td>60.5 (44–72)</td>
<td>56.5 (46–80)</td>
</tr>
<tr>
<td>Duration symptoms, (months)</td>
<td>50.2</td>
<td>24.8</td>
<td>6.6 ± 2.5</td>
<td>6.6 ± 2.9</td>
<td>NA</td>
</tr>
<tr>
<td>Diagnostic delay (months)</td>
<td>24.4</td>
<td>0.9</td>
<td>0.027 ± 0.016</td>
<td>0.020 ± 0.010</td>
<td>NA</td>
</tr>
<tr>
<td>Disease progression rateb</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</td>
<td>98.5 ± 17.5</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 ± 4.6</td>
<td>40.1 ± 4.7</td>
<td>40.1 ± 3.9</td>
<td>41.8 ± 3.7c</td>
<td>48.0 ± 0</td>
</tr>
<tr>
<td>Finger tapping/s²c</td>
<td>2.3 ± 1.2</td>
<td>4.0 ± 1.3</td>
<td>3.3 ± 1.3</td>
<td>4.8 ± 0.7</td>
<td>5.1 ± 0.6</td>
</tr>
<tr>
<td>Mean fractional anisotropy corticospinal tract</td>
<td>0.42 ± 0.02</td>
<td>0.42 ± 0.03</td>
<td>0.43 ± 0.03</td>
<td>0.46 ± 0.02</td>
<td>0.45 ± 0.02</td>
</tr>
</tbody>
</table>

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).

a Defined as a score of 3 or lower on at least two of the three bulbar items of the ALSFRS-R (speech, sialorrhoea and swallowing) at baseline.

b Disease progression rate = (ALSFRS-R score at baseline)/disease duration (days).

c Finger tapping = (left + right)/2.

### Table 2 Clinical characteristics and mean fractional anisotropy changes over time (6 months)

<table>
<thead>
<tr>
<th></th>
<th>Follow-up Bulbar-onset ALS (n = 9) baseline</th>
<th>Follow-up Limb-onset ALS (n = 7) baseline</th>
<th>Follow-up PMA (n = 10) baseline</th>
<th>Follow-up Controls (n = 11) baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Revised El Escorial classification a/b/c</td>
<td>0/7/2</td>
<td>1/6/0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>No. of patients with bulbar symptoms</td>
<td>9</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Vital capacity (%)</td>
<td>97.7 ± 15.7c</td>
<td>106.9 ± 19.3</td>
<td>101.0 ± 16.0</td>
<td>91.3 ± 23.3</td>
</tr>
<tr>
<td>ALSFRS-R</td>
<td>41.7 ± 3.4c</td>
<td>42.6 ± 2.0</td>
<td>42.8 ± 3.5</td>
<td>41.9 ± 3.5</td>
</tr>
<tr>
<td>Finger tapping speed (/s)</td>
<td>4.2 ± 1.2c</td>
<td>3.3 ± 1.3</td>
<td>4.8 ± 0.8</td>
<td>4.8 ± 0.9</td>
</tr>
<tr>
<td>Mean fractional anisotropy corticospinal tract</td>
<td>0.43 ± 0.03</td>
<td>0.44 ± 0.03</td>
<td>0.46 ± 0.02</td>
<td>0.45 ± 0.02</td>
</tr>
</tbody>
</table>

Data are given as mean ± SD, unless otherwise indicated. Values at follow-up are shown in italics. Statistical significance based on Wilcoxon test for paired data, threshold set at P < 0.05 (P-values in superscript). NA = not applicable.
At follow-up, mean fractional anisotropy of the corticospinal tract in limb-onset ALS was significantly lower \((P = 0.018)\) when compared with 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 (Fig. 2A).

In bulbar-onset ALS, fractional anisotropy was significantly lower compared with controls in the medulla oblongata, caudal pons, cerebral peduncle and posterior limb of the internal capsule (Fig. 2B).

In limb-onset ALS, fractional anisotropy was significantly lower compared with controls in the medulla oblongata and in the subcortical white matter (Fig. 2C).

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 (Fig. 2D).

**Comparisons between patient groups at baseline**

In PLS, fractional anisotropy was significantly lower in the distal medulla oblongata when compared with limb-onset ALS, whereas fractional anisotropy was higher in the rostral part of the posterior limb of the internal capsule, and lower in the subcortical white matter when compared with bulbar-onset ALS. 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 bulbar-onset ALS, fractional anisotropy was significantly lower in the medulla oblongata, pons, posterior limb of the internal capsule and corona radiata when compared with PMA. A comparison between limb-onset ALS and bulbar-onset ALS yielded no significant differences.

In limb-onset ALS, fractional anisotropy was significantly lower in the pons, rostral posterior limb of the internal capsule and subcortical white matter when compared with PMA.

**Findings at follow-up**

In bulbar-onset ALS, 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 (Fig. 3A).

In limb-onset ALS, 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 (Fig. 3B).

In PMA, fractional anisotropy increased significantly over time in the subcortical white matter (Fig. 3C).

No significant fractional anisotropy changes were found in the controls over time (Fig. 3D).

**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 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 (Fig. 4A and Table 3).

At baseline, a significant fractional anisotropy decrease in patients with bulbar-onset ALS 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 (Fig. 4B and Table 3).

When comparing patients with limb-onset ALS to 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 (Fig. 4C and Table 3).

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 (Fig. 4D and Table 3).

**Comparisons between patient groups at baseline**

Fractional anisotropy was significantly lower in PLS compared with bulbar-onset ALS 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 (Supplementary Fig. 2A and Supplementary Table 1).

When comparing PLS to limb-onset ALS 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 (Supplementary Fig. 2B and Supplementary Table 1).

In a direct comparison of PLS and patients with 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 (Supplementary Fig. 2C and Supplementary Table 1).

Fractional anisotropy was significantly lower in bulbar-onset ALS compared with limb-onset ALS 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 (Supplementary Fig. 2D and Supplementary Table 1).

The direct comparison between bulbar-onset ALS and PMA at baseline yielded significantly lower fractional anisotropy in the patients with bulbar-onset ALS 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 bulbar-onset ALS (Supplementary Fig. 2E and Supplementary Table 1).

When comparing limb-onset ALS directly to PMA at baseline, significantly lower fractional anisotropy values were observed in the patients with limb-onset ALS 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 limb-onset ALS compared with PMA (Supplementary Fig. 2F and Supplementary Table 1).

Findings at follow-up
When comparing the baseline and follow-up scans of nine patients with bulbar-onset ALS, 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

respectively. Asterisks and hash signs indicate locations in which statistically significant differences between patient group and controls were found (Mann–Whitney U-test, \(* P < 0.01, ^# P < 0.05\)). The middle image provides an anatomic reference. ALS-B = bulbar-onset ALS; ALS-L = limb-onset ALS; CP = cerebral peduncle; CR = corona radiata; MO = medulla oblongata; P = pons; PLIC = posterior limb of the internal capsule; SWM = subcortical white matter.
thalamus, the hippocampal formations and the right cingulum (Fig. 5A and Table 3).

When comparing the baseline and follow-up scans of seven patients with limb-onset ALS, 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 (Fig. 5B and Table 3).

When comparing fractional anisotropy over time in 10 patients with PMA, a pattern 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 (Fig. 5C and 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 motoneuron 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 fibres tend to fan out at the more cranial levels of the corticospinal tract. These varying degrees of fibre coherence and 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 (Sage et al., 2007), 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 PLS. 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.
Figure 4 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 with 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). CST = corticospinal tract; CC = corpus callosum; IC = internal capsule; L = left; R = right.
First, it should be noted that a direct comparison of different studies applying diffusion tensor tractography or voxel-based analysis in motoneuron disease phenotypes is not trivial. A factor hindering objective comparison is that in most previous studies, varying proportions of limb-onset ALS, bulbar-onset ALS, PLS or patients with PMA were included. It is unclear to what 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 (Jones et al., 2005; Zhang et al., 2007; Sage et al., 2009). 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 motoneuron 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 (Mitchell et al., 2010).

A limitation of our fibre tracking study is that we calculated multiple P-values that 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 $P \leq 0.001$ uncorrected to avoid underestimation of effects in our relatively small cohorts. Moreover, the same statistical threshold was adopted in a previous voxel-based analysis study in patients with ALS (Agosta et al., 2007), in which the threshold was chosen according to previous reports (Abe et al., 2004; Kassubek et al., 2005) and to a priori hypothesis based on available studies (Sach et al., 2004; Sage et al., 2007).

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**Table 3** Baseline and follow-up voxel-based analysis data of four cohorts of motoneuron disease phenotypes

<table>
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<tr>
<th></th>
<th>PLS Baseline n = 12</th>
<th>Bulbar-onset ALS Baseline n = 12</th>
<th>Over time n = 9</th>
<th>Limb-onset ALS Baseline n = 12</th>
<th>Over time n = 7</th>
<th>PMA Baseline n = 12</th>
<th>Over time n = 10</th>
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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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<td>WM underneath primary motor cortex</td>
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<td>Thalamus</td>
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<tr>
<td>Cingulum</td>
<td>R</td>
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<td>+</td>
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<td>Genu internal capsule</td>
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<td>Medial cerebellar peduncle</td>
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<td>WM of angular gyrus</td>
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Locations of fractional anisotropy reductions in patients when compared with controls at baseline, and when comparing patient data pair-wise over time. L = difference present in left hemisphere; R = difference present in right hemisphere; B = different present in both hemispheres; + = difference present in a midline structure; WM = white matter; CC = corpus callosum.

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**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 motoneuron disease phenotypes is not trivial. A factor hindering objective comparison is that in most previous studies, varying proportions of limb-onset ALS, bulbar-onset ALS, PLS or patients with PMA were included. It is unclear to what 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 (Jones et al., 2005; Zhang et al., 2007; Sage et al., 2009). 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 motoneuron 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 (Mitchell et al., 2010).

A limitation of our fibre tracking study is that we calculated multiple P-values that 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 $P \leq 0.001$ uncorrected to avoid underestimation of effects in our relatively small cohorts. Moreover, the same statistical threshold was adopted in a previous voxel-based analysis study in patients with ALS (Agosta et al., 2007), in which the threshold was chosen according to previous reports (Abe et al., 2004; Kassubek et al., 2005) and to a priori hypothesis based on available studies (Sach et al., 2004; Sage et al., 2007).
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 (Wang et al., 2006; Wong et al., 2007). This may be explained by the fact that the clinical side of onset is also and sometimes predominantly determined by lower motoneuron involvement.

**Primary lateral sclerosis**

**Corticospinal tract**

We demonstrated lower fractional anisotropy along the entire course of the corticospinal tract in patients with PLS when compared with controls in both the diffusion tensor tractography 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 with controls in a region of interest analysis (Ulug et al., 2004). A recent voxel-based analysis reported more widespread fractional anisotropy reduction in a cohort of 25 patients with PLS, which resembled our observations (Unrath et al., 2010).

In direct comparisons between the motoneuron 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 six patients with PLS were reported (mean disease duration 7.4 years) when compared with patients with ALS (Ciccarelli et al., 2009). This suggests more advanced corticospinal tract degeneration in PLS than in other patient groups, which may be explained by the upper motoneuron nature of PLS in combination with 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 study. This may be understood as PLS is clinically at the upper motoneuron end of the motoneuron disease spectrum and PMA at the lower motoneuron end. When comparing PLS and bulbar-onset ALS with 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 bulbar-onset ALS, this decrease seemed to extend slightly more rostrally in bulbar-onset ALS 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, 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 similar to our findings (Ciccarelli et al., 2009; Unrath et al., 2010). Likewise, reduction of white matter volume and $^{11}$C-flumazenil binding were recently reported in similar areas of the motor cortex and corpus callosum (Turner et al., 2007; Tartaglia et al., 2009). Also when compared with other motoneuron 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 corticospinal tract that connect the motor cortices that was described earlier (Brownell et al., 1970; Ciccarelli et al., 2009). 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 fibres connecting single functional cortical areas (Thompson et al., 2002). 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 (Tartaglia et al., 2009), 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 (Kuipers-Upmeijer et al., 2001; Turner et al., 2007).

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 (Davidoff, 1990). 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 but 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 (Caselli et al., 1995). 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 (Behrens et al., 2003; Unrath et al., 2008) and which could be related to functional anatomical divisions within the thalamus in a probabilistic atlas (Johansen-Berg et al., 2005). 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 limb-onset ALS and bulbar-onset ALS to controls at baseline, the diffusion tensor tractography and voxel-based analysis studies yielded similar results, with the fractional anisotropy reduction in limb-onset ALS 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 bulbar-onset ALS 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 limb-onset ALS and bulbar-onset ALS in the diffusion tensor tractography study, but significantly lower fractional anisotropy in the caudal parts of the corticospinal tract in
bulbar-onset ALS in the voxel-based analysis study when comparing these patient groups directly. Differing extent of upper motor-neuron involvement between limb-onset ALS and bulbar-onset ALS cannot be explained by differing disease severity or duration between the groups, as these were similar.

Only few previous diffusion tensor imaging studies assessed patients with limb-onset ALS and bulbar-onset ALS as separate groups, as in most studies patients with limb-onset ALS and bulbar-onset ALS were pooled to obtain a single study population (Sach et al., 2004; Agosta et al., 2007; Sage et al., 2007, 2009), or no information was provided on the onset type (Graham et al., 2004; Wang et al., 2006; Zhang et al., 2007; Ciccarelli et al., 2009; Stanton et al., 2009). Moreover, most of these studies focused on (parts of) the corticospinal tract by either performing region of interest analyses (Ellis et al., 1999; Graham et al., 2004; Mitsumoto et al., 2007; Senda et al., 2009) or fibre tracking (Aoki et al., 2005; Wang et al., 2006; Sage et al., 2007; Senda et al., 2009). Lower fractional anisotropy in the corticospinal tract of patients with bulbar-onset ALS compared with controls (Ellis et al., 1999; Aoki et al., 2005), and lower mean fractional anisotropy in bulbar-onset ALS compared with limb-onset ALS (Ellis et al., 1999) were observed in previous studies, although the latter was not confirmed (Aoki et al., 2005).

A limited number of studies performed spatial profiling along the corticospinal tract, none of which included separate groups of patients with limb-onset ALS and bulbar-onset ALS. 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 patients with ALS 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 ± 12 months were found (Sage et al., 2007; Wong et al., 2007). 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 (Senda et al., 2009). Other studies comparing sporadic patients with ALS to controls using voxel-based analysis or tract-based spatial statistics reported more extensive patterns of fractional anisotropy reductions within the corticospinal tract of patients with ALS at a more advanced stage of the disease (Sach et al., 2004; Agosta et al., 2007; Sage et al., 2007, 2009; Ciccarelli et al., 2009; Stanton et al., 2009).

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 (Blain et al., 2007; Mitsumoto et al., 2007; Sage et al., 2007). However, in the voxel-based analysis study, significant fractional anisotropy reductions over time were observed throughout the corticospinal tract in both limb-onset ALS and bulbar-onset ALS, 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 patients with sporadic limb-onset ALS 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 thalamus (Sach et al., 2004; Agosta et al., 2007; Sage et al., 2007, 2009; Ciccarelli et al., 2009; Stanton et al., 2009). The disease duration of the patients with limb-onset ALS in these studies was generally longer than that of our patients with limb-onset ALS (10.5 ± 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 limb-onset ALS and bulbar-onset ALS in this study and compared with controls in previous studies (Sach et al., 2004; Agosta et al., 2007; Ciccarelli et al., 2009; Sage et al., 2009) might relate to the observation of impaired transcallosal inhibition and mirror movements in a number of patients with ALS (Wittstock et al., 2007; Bartels et al., 2008), as anatomically, the central part of the corpus callosum contains inter-hemispheric fibres between the motor cortices.

The reduced fractional anisotropy over time in the genu of the corpus callosum in bulbar-onset ALS may be related to the cognitive impairment that is often reported in patients with ALS (Phukan et al., 2007; Raaphorst et al., 2010), 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 (Yamauchi et al., 1995), although it is unclear whether this study population also included patients with bulbar-onset ALS. It has been suggested that cognitive impairment may be more prevalent in patients with bulbar-onset ALS than in patients with limb-onset ALS (Abrahams et al., 1997; Lomen-Hoerth et al., 2003; Schreiber et al., 2005), although this finding was not confirmed in other studies (Raaphorst et al., 2010). In our study, different structures involved in cognition and memory were impaired in patients with bulbar-onset ALS, whereas in patients with limb-onset ALS, 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 bulbar-onset ALS than in those with limb-onset ALS. 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 bulbar-onset ALS when compared with controls, two frontal areas involved in executive function (Tanji and Hoshi, 2008) and working memory (Linden, 2007). The inferior frontal gyrus plays an important role in verbal fluency, which is most frequently reported impaired in patients with ALS (Abrahams et al., 1997; Phukan et al., 2007; Raaphorst et al., 2010). A recent meta-analysis showed a relation between visual and verbal memory impairments in patients with ALS and frontal lobe involvement (Raaphorst et al., 2010). In this view, the
fractional anisotropy reductions in the hippocampal formations and cingulum over time in the patients with bulbar-onset ALS and in the frontal white matter in patients with limb-onset ALS 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 limb-onset ALS to controls (Sage et al., 2007, 2009; Ciccarelli et al., 2009).

When comparing bulbar-onset ALS directly to other motoneuron disease phenotypes, the most striking observation was that fractional anisotropy in the temporal white matter of the patients with bulbar-onset ALS was lower than in any of the other phenotypes. The white matter underneath the inferior temporal gyrus was also affected in limb-onset ALS when compared directly with 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 (Phukan et al., 2007; Raaphorst et al., 2010).

Fractional anisotropy was reduced within the genu of the internal capsule and the lateral precentral gyrus of patients with bulbar-onset ALS 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 bulbar-onset ALS may provide evidence for impairment of the corticobulbar tracts in bulbar-onset ALS, as pseudobulbar affect was observed in seven and nine patients at baseline and at follow-up, respectively.

Finally, in both patients with bulbar-onset ALS and limb-onset ALS, we demonstrated reduced fractional anisotropy in the thalamus when compared with controls. In patients with bulbar-onset ALS, this fractional anisotropy reduction was located in the part of the ventral anterior nucleus projecting to the prefrontal cortices as described in a previous study (Johansen-Berg et al., 2005), which is similar to the location in which we found a fractional anisotropy reduction in patients with PLS compared with controls. This may again contribute to the cognitive impairment that is often reported in ALS.

Conversely, in the patients with limb-onset ALS, 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 (Johansen-Berg et al., 2005). Together with the fractional anisotropy changes in ALS in the white matter underneath primary somatosensory cortex, premotor cortex and superior parietal lobe, this may represent secondary degeneration of tracts involved in voluntary motor control (Sage et al., 2007).

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 fractional anisotropy 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 fibres is indicative of corticospinal tract degeneration, as was described previously in ALS (Wong et al., 2007) and other neurodegenerative diseases (Ciccarelli et al., 2001; Klöppel et al., 2008; Douad et al., 2009), 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 motoneuron 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 limb-onset ALS and in the caudal and middle section of the corticospinal tract in bulbar-onset ALS. 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 (Cosottini et al., 2005; Mitsumoto et al., 2007), whereas another study demonstrated decreased fractional anisotropy in the rostral section of the corticospinal tract in patients with a lower motoneuron phenotype (Sach et al., 2004). However, all patients of the latter study developed upper motoneuron signs in a later course of their disease, whereas none of our patients had clinical evidence of upper motoneuron 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 (Graham et al., 2004). 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 motoneuron signs during life (Brownell et al., 1970; Norris, 1991; Ince et al., 2003).

Extra-motor findings

The fractional anisotropy reduction in the genu of the internal capsule 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 frontal 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 (Gallassi et al., 1985), which could not be confirmed in a more recent study (Wicks et al., 2006). Another recent study found impaired category fluency and working memory in these patients (Raaphorst et al., 2009). 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 over time 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 motoneuron disease and the El Escorial classification criteria

In this study, we demonstrated both upper motoneuron and extra-motor white matter involvement in all phenotypes of motoneuron disease, which was progressive in limb-onset ALS, bulbar-onset ALS 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 anatomical distribution and even the spread of white matter involvement in the different motoneuron 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 ‘motoneuron 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 (Brooks et al., 2000). This classification is based on the revised El Escorial criteria, which are based on clinical evidence of upper motoneuron in combination with lower motoneuron involvement, and actually exclude patients with PLS or PMA.

### Spread of pathology in motoneuron 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 motoneuron, which recruits the lower motoneuron into the degenerative process through an anterograde ‘dying forward’ process (Eisen et al., 1992; Eisen, 1995; Eisen and Weber, 2001). In contrast, others proposed that the onset of neuronal degeneration in ALS is targeted at the lower motoneuron level, with spreading to the upper motoneuron by a retrograde or ‘dying-back’ process (Chou and Norris, 1993). 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 motoneuron and lower motoneuron 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 (Ravits et al., 2007). Since then, dual focality of onset (cortex and anterior horn) with contiguous spread outwards from these foci in both lower and upper motoneuron pathways is one of the most widely supported views of ALS pathogenesis (Ravits and La Spada, 2009).

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 motoneuron degeneration, as we only obtained diffusion tensor imaging data at the upper motoneuron level and our patients with limb-onset ALS and bulbar-onset ALS had, by definition, clinical evidence of both upper and lower motoneuron degeneration at the time of scanning. The observed differences in the distribution and extent of upper motoneuron involvement between bulbar-onset ALS and limb-onset ALS 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 limb-onset ALS, 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 limb-onset ALS, bulbar-onset ALS and PMA appeared rather similar, especially in limb-onset ALS and PMA. These results are in line with the view of contiguous outward spread (Ravits and La Spada, 2009). Although further study is required with more subjects per motoneuron disease phenotype to validate our findings, our study shows that diffusion tensor imaging can be a valuable tool in evaluating the upper motoneuron and extra-motor involvement at early stages of the disease and the spread of pathology over time.

### Conclusion

Upper motor and extra-motoneuron involvement were observed in all phenotypes of motoneuron 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 motoneuron disease, and for monitoring the spread of pathology over time.
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Supplementary material

Supplementary material is available at Brain online.

References

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