ORIGINAL CONTRIBUTION

Fractional Anisotropy in the Posterior Limb of the Internal Capsule and Prognosis in Amyotrophic Lateral Sclerosis

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Objective: To explore the value of diffusion tensor imaging applied to those specific cerebral white matter tracts consistently involved pathologically in amyotrophic lateral sclerosis as a source of prognostic biomarkers.

Design: Baseline clinical assessment and 3-T diffusion tensor imaging, repeated after approximately 6 months. Tract-based spatial statistics were used to assess voxelwise correlations of just the baseline diffusion tensor imaging indices with the progression rate (change in disability score/time interval) within the corticospinal tract and corpus callosum.

Patients: The study involved 21 patients with amyotrophic lateral sclerosis and 3 patients with primary lateral sclerosis.

Results: Correlation was observed between fractional anisotropy and progression rate for a region of the corticospinal tract spanning the posterior limb of the internal capsule, with a left hemisphere emphasis. Posterior limb of the internal capsule fractional anisotropy showed potential to distinguish those patients with rapid progression. Axial diffusivity significantly increased in this region in a paired *t* test analysis of baseline and follow-up diffusion tensor imaging, in keeping with axonal damage. No correlations were noted for the corpus callosum.

Conclusions: Posterior limb of the internal capsule fractional anisotropy is a candidate prognostic marker in amyotrophic lateral sclerosis, with potential to identify incident cases with more rapid progression.

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THE HETEROGENEITY OF PROGnosis in the progressive and fatal neurodegenerative disorder amyotrophic lateral sclerosis (ALS) is recognized as a major challenge to streamlining therapeutic trials.[1] Biomarkers sensitive to prognosis would help to optimize care planning and decision making in the current era of palliative-led care for ALS, and they would also permit stratification of patients within therapeutic trials.[2] Such biomarkers should ideally be sensitive to the range of clinical phenotypes encompassed by the wider-term motor neuron disease, which includes the rare upper motor neuron (UMN)–only condition primary lateral sclerosis (PLS), which has a uniformly slow progression rate.

Magnetic resonance imaging (MRI) as a potential source of biomarkers fulfills several desirable criteria. It is a ubiquitous, noninvasive technology, part of the routine diagnostic workup, and is generally tolerated by most patients in the first half of their disease course.[3] The development of diffusion tensor imaging (DTI) has permitted the study of white matter integrity in vivo, with obvious application to diseases of long tracts such as ALS.[4] Although several parameters can be derived from the diffusion tensor within each voxel, fractional anisotropy (FA) has been the most consistently applied to ALS, decreasing with loss of tract integrity. Fractional anisotropy is derived from the eigenvalues of the diffusion tensor, which assesses diffusion in 3 orthogonal directions: L1, L2, and L3.

In the decade since the first application of DTI to ALS,[5] multiple studies have demonstrated reduced FA within (though not confined to) the corticospinal tract (CST) in patients with ALS (see review by Turner et al[2]). The corticospinal tracts and interhemispheric motor cortical fibers of the corpus callosum (CC) have been most consistently involved in ALS.[6,7] Patients with PLS show similar, more marked changes.[8,9]

Longitudinal studies in ALS have demonstrated a range of FA reductions over

time.[10-13] In 2 cross-sectional studies, greater FA values were noted among those patients with the longest disease durations,[6,9] with the possibility that absolute FA values might have inherent prognostic value. In a survival analysis using whole CST FA measurement, lower values were a significantly adverse covariate.[14]

This DTI study sought to identify the parts of the CST or CC that might be most sensitive to progression rate across a range of phenotypes (including some cases of PLS) using tract-based spatial statistics (TBSS).

METHODS

PARTICIPANTS AND CLINICAL MEASURES

Patients were recruited from the Oxford Motor Neuron Disease Care & Research Centre, having been diagnosed as having ALS[15] or PLS[16] by 2 experienced neurologists (M.R.T. and K.T.). Ethical approval for all procedures was obtained (South Central Oxford Ethics Committee reference number: 08/H0605/85).

Patients were clinically examined on the day of MRI and again after an approximate 6-month interval by the same person (M.R.T.). The functional status of all patients was assessed using the revised ALS Functional Rating Scale (ALSFRS-R), in which a lower score reflects greater disability.[17] The difference between the 2 scores for each patient was divided by the exact number of days between the baseline and follow-up assessment to calculate a rate of ALSFRS-R score change, synonymous in this article with progression rate. To investigate longitudinal changes of white matter microstructural integrity, MRI was repeated on the day of the 6-month follow-up assessment.

There was some overlap in the patients with ALS (but not PLS) in a previously published DTI study from our group.[6]

IMAGE ACQUISITION AND ANALYSIS

Whole-brain DTI images were acquired with a 3-T Siemens Trio MR scanner (Siemens) with a 12-channel head coil using an echoplanar imaging sequence (60 isotropic directions; b value = 1000 seconds/mm^2; echo time/repetition time = 94 ms/10 000 ms; $2 \times 2 \times 2$ mm^3 voxel size; 65 slices). In addition, 4 images without diffusion weighting were acquired. Furthermore, a field map was acquired using a gradient echo imaging sequence ($2 \times 2 \times 2$ mm^3 voxel size; 65 slices; echo time 1/echo time 2/repetition time = 5.19 ms/7.65 ms/655 ms) to account for distortions caused by field inhomogeneities.

All images were analyzed using the Oxford Centre for Functional Magnetic Resonance of the Brain software library tools.[18] All scans were corrected for head motion and eddy currents and then brain extracted to remove any nonbrain voxels. To correct for B0 inhomogeneities and unwarp scans, field map correction was performed with FUGUE software. Fractional anisotropy, mean diffusivity (MD), L1, L2, and L3 maps were created using DTIFIT software by applying a diffusion tensor model to each voxel.[19] Individual FA images of all subjects were nonlinearly registered to a standard FA template (http://www.fmrib.ox.ac.uk/fsl/data/FMRIB58_FA), and then averaged to create a study-specific template to which each subject's FA map was then nonlinearly registered. The same transformation that was used to register the individual FA images to the study-specific template was subsequently applied to register the individual MD, L1, L2, and L3 images.

REGIONS OF INTEREST

The Juelich Histological Atlas was used to produce masks of the left and right CST in Montreal Neurological Institute standard space (threshold, 25). The CC mask was taken from the Johns Hopkins University ICBM-DTI-81 White-Matter Labels Atlas.

TBSS ANALYSES

Tract-based spatial statistics analyses were carried out to assess voxelwise correlations between baseline DTI indices and the rate of change of ALSFRS-R scores, as well as the baseline ALSFRS-R scores and disease duration at baseline.[20] A permutation-based nonparametric inference within the framework of the general linear model (5000 permutations) was used to achieve accurate inference in the voxelwise analyses, including correction for multiple comparisons within each region of interest (ROI).[21] Nonparametric tests were used to safeguard against the possibility that the between-subjects effects were non-Gaussian. Tract-based spatial statistics results for FA, MD, and related DTI eigenvalues were considered significant for $P < .05$ (corrected for multiple comparisons [familywise error (FWE)] and age), using the 2-dimensional parameter settings with threshold-free cluster enhancement.[22] Paired *t* tests within TBSS were used to perform a longitudinal analysis of DTI measures.

SCATTERPLOTS

To qualitatively explore and graphically illustrate the relationship between significantly correlated DTI measures and progression rate in individual subjects, a binary mask was created from the significant TBSS results. The respective voxel values averaged over all voxels within this mask were extracted for each patient and plotted against the progression rate. A median rate of progression was estimated to be 0.053 ALSFRS-R units per day based on the generally accepted median survival in ALS of 30 months from symptom onset.[23]

RESULTS

Twenty-four patients were recruited, 21 with ALS and 3 with PLS. Demographic and clinical parameters are shown in the **Table**. Mean (SD) age at baseline scan was 60 (12) years (range, 39-83 years). Mean (SD) disease duration (symptom onset to baseline scan) was 65 (54) months (range, 10-247 months). Mean (SD) ALSFRS-R score at baseline was 34 (4) (range, 26-43), with a mean (SD) change in score of 4 (6) (range, 0-25). The mean (SD) assessment interval was 193 (19) days (range, 154-222 days). There was no significant difference in progression rate for bulbar-onset vs limb-onset patients in our cohort.

TBSS ROI ANALYSIS

Tract-based spatial statistics ROI analysis included the following measures and outcomes.

1. Baseline DTI measures vs baseline ALSFRS-R score: No significant correlation was found for either ROI.
2. Baseline DTI measures vs baseline disease duration: A positive correlation with the eigenvalue L3 was seen in the right upper CST.
3. Baseline DTI measures vs rate of progression: A region was identified spanning the PLIC bilaterally in which

Table. Clinical and Demographic Patient Features

Patient Sex/ Age at MRI, y	Diagnosis	Site of Onset	EE Category	Disease Duration, mo	Baseline ALSFRS-R Score	Change in ALSFRS-R Score	Assessment Interval, d
M/69	ALS	R UL	Possible	32	33	1	189
F/67	ALS	BO	Possible	62	33	4	191
M/76	ALS	BO	Possible	43	33	4	210
F/54	ALS	R LL	Definite	38	36	4	176
F/47	ALS	R LL	Definite	122	34	2	217
M/53	ALS	R UL	Probable	24	26	3	219
M/69	ALS	L UL	Possible	33	39	5	161
M/67	ALS	BO	Definite	10	32	25	154
F/48	ALS	R UL	Possible	73	33	1	196
F/55	ALS	L LL	Probable	78	37	1	196
M/59	ALS	L LL	Probable	21	37	3	191
M/69	ALS	R UL	Possible	78	34	4	215
F/55	ALS	L LL	Probable	28	35	13	189
M/39	ALS	L UL	Definite	22	35	3	168
M/63	ALS	L LL	Suspected[a]	75	30	1	209
M/41	ALS	R UL	Definite	79	36	1	191
M/60	ALS	R UL	Possible	121	28	1	210
M/83	ALS	R UL	Possible	107	32	1	182
M/50	ALS	BO	Probable	13	36	11	161
M/65	ALS	BO	Possible	29	43	0	208
F/46	ALS	R LL	Definite	20	32	14	222
F/76	PLS	BO	Possible	143	32	2	175
F/64	PLS	L LL	Possible	247	29	1	194
F/76	PLS	Legs	Possible	56	37	2	196

Abbreviations: ALS, amyotrophic lateral sclerosis; BO, bulbar onset; EE, El Escorial; F, female; FRS, Functional Rating Scale; L, left; LL, lower limb; M, male; MRI, magnetic resonance imaging; PLS, primary lateral sclerosis; R, right; UL, upper limb.
[a]Evolved to possible.

there was significant correlation between baseline FA and the rate of ALSFRS-R score change ($P < .05$, FWE and age corrected). With correction for disease duration, correlation within the left PLIC remained significant (**Figure 1**). Positive correlations were also found for the eigenvalue L3 and MD in the left CST, which persisted with the addition of correction for disease duration (not shown).

No significant correlations were observed between DTI measures and rate of progression for the CC ROI.

TBSS LONGITUDINAL ANALYSIS

Nineteen of the 24 patients had a useable second MRI for additional longitudinal analysis of DTI measures (5 data sets had to be discarded owing to spike artifacts). Paired *t* test analysis revealed higher axial diffusivity (eigenvalue L1) at follow up in the region of the left PLIC (**Figure 2**). There was no significant change in the other eigenvalues, FA, or MD.

COMMENT

This study demonstrated that FA is correlated with progression rate within a specific region of the CST spanning the PLIC, and across a range of phenotypes including patients with PLS and some patients with ALS with few UMN signs clinically. In a longitudinal analysis of interval scans, there was corroborative evidence of axonal damage (namely increased axial diffusivity) in this region.

Only 4 patients lay above the median rate of progression in our study, with a marked spread of FA values in those below the median. Therefore, the prognostic value of PLIC FA may be limited to identifying rapidly progressing cases below a cutoff value (<0.62 in this study), similar to the observation in a previous survival analysis that used whole CST FA as a covariate.[14] Nonetheless, early identification of such individuals would have clear value in care planning and potentially also stratification of patients in therapeutic trials.

CST FA AND DISABILITY IN ALS

Multiple cross-sectional studies using DTI in ALS have noted FA decreases within 1 or more CSTs compared with healthy control subjects, some noting correlations with disability at the time of the scan.[5,24,25] However, a volumetric DTI analysis of the CST did not reveal any regional correlations with absolute ALSFRS-R values (or other clinical measures).[26] In a small longitudinal study involving 7 patients with ALS with prominent UMN signs (ie, El Escorial probable and definite categories), a correlation was noted between CST FA and absolute ALSFRS-R scores at the time of the scans ($R^2 = 0.64$),[11] but progression rate was not studied.

PLIC IN ALS

Involvement of the PLIC was specifically noted in post mortem study of cerebral white matter tract degenera-

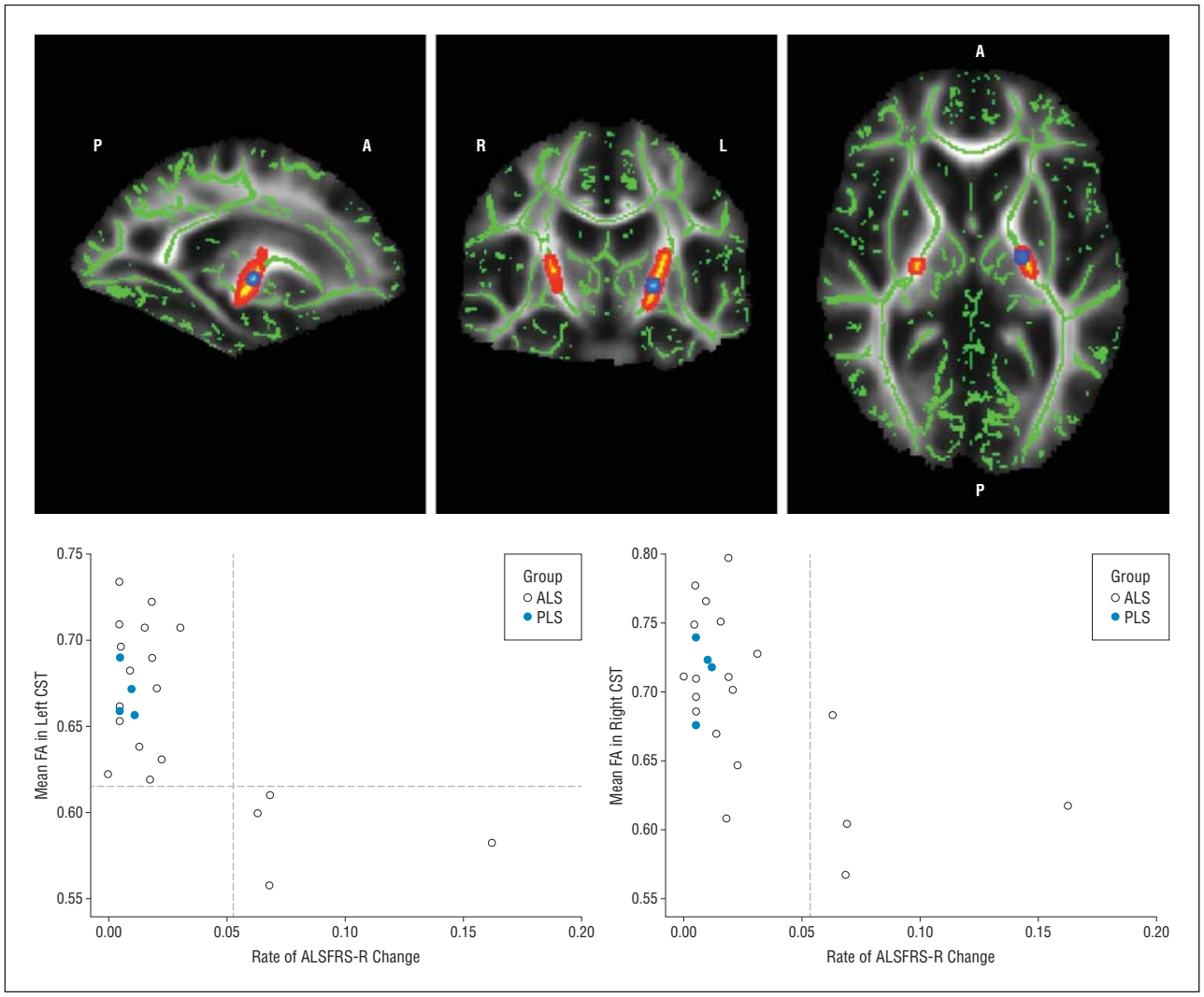

Figure 1. Tract-based spatial statistics results within the corticospinal tract (CST) in relation to progression rate. Significant tract-based spatial statistics results ($P < .05$, familywise error corrected) in sagittal, coronal, and axial views (top panel) overlaid onto the group's mean fractional anisotropy (FA) skeleton (green) and the FMRIB58 FA template showing those parts of the CSTs where baseline FA was associated with progression rate (age included as a covariate of no interest). This revealed a bilateral region through the posterior limb of the internal capsules (red). The left side remained correlated when disease duration was added as a further potential confounding variable (blue). Scatterplots are shown for average FA within the tract-based spatial statistics–significant masks in the left and right CST vs progression rate between the 2 points to demonstrate the correlation in this region (bottom panel; estimates subject to circularity bias). The dotted vertical line shows the median progression rate based on a median survival of 30 months from symptom onset in amyotrophic lateral sclerosis (ALS). The horizontal dotted line in the left posterior limb of the internal capsule shows that baseline FA less than 0.62 identified the 4 patients above the median in this study. A indicates anterior; FRS, Functional Rating Scale; L, left; P, posterior; PLS, primary lateral sclerosis; R, right.

tion in ALS.[27] Hyperintensities within the PLICs were noted on one of the first MRI studies in ALS,[28] and specifically on proton-density images.[14] Intensity changes within this region are a common observation on routine clinical MRI studies during the diagnostic workup of patients with ALS, but they lack sensitivity.[29,30]

Tractography studies have shown that the PLIC maps to motor and premotor cortices,[31] which is entirely in keeping with the burden of cerebral pathology in ALS. Posterior limb of the internal capsule FA has been previously linked broadly to clinical disease burden in ROI-based DTI analysis[32] and highlighted in spectroscopy studies.[33,34] Posterior limb of the internal capsule involvement was also a consistent feature in the meta-analysis of 8 DTI studies in ALS.[35] An attempt to assess corticobulbar pathway involvement using DTI used a region including the PLIC and was shown to have lower FA values in bulbar-onset patients.[36] We did not note a significant difference in our cohort for progression rate and site of onset, although this may simply reflect the small sample size.

Reduced PLIC FA was noted in a DTI study of healthy presymptomatic subjects at risk for ALS through autosomal dominant mutations of the superoxide dismutase-1 (*SOD1*) gene,[37] suggesting this region may even have sensitivity to the preclinical pathologic changes in ALS.

DISEASE DURATION AND APPARENT LATERALIZATION

Our patient group contained patients of relatively long disease duration. By definition, such patients have a slower rate of progression. Curve estimation for disease duration plotted against progression rate confirmed an inverse relationship in our cohort, although importantly

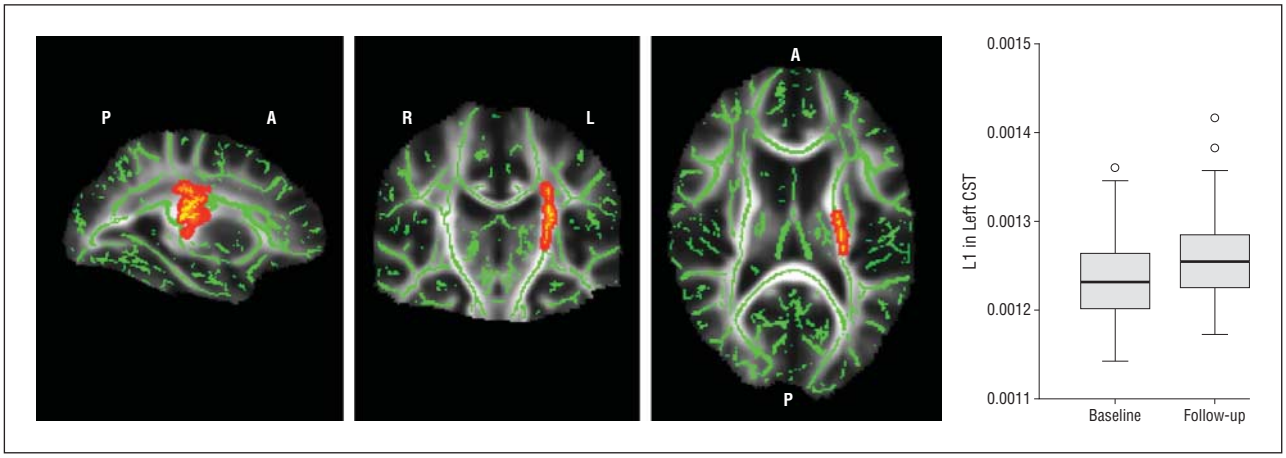

Figure 2. Longitudinal tract-based spatial statistics changes within the corticospinal tract (CST). Significant tract-based spatial statistics paired *t* test results ($P < .05$, familywise error corrected) in sagittal, coronal, and axial views (left 3 panels) overlaid onto the group's mean fractional anisotropy skeleton (green) and the FMRIB58 fractional anisotropy template showing those parts of the CSTs where the L1 (axial diffusivity) values significantly increased between the baseline and follow-up scans. This suggests left posterior limb of the internal capsule region axonal damage. Box plot also shown (far right panel). A indicates anterior; L, left; P, posterior; R, right.

there was no correlation between baseline ALSFRS-R score and disease duration (data not shown). Patients with more benign disease tend to present much later to specialist clinics,[38] leading to an inherent bias in many ALS research studies whereby the cases with the shorter disease durations are invariably also the most rapidly progressive. Thus, disease duration must always be considered as a potential confound.

Prior to the inclusion of disease duration as an additional covariate, the area of significant FA reduction was bilateral in our cohort but became restricted to only the left CST thereafter. The longitudinal DTI changes were also restricted to the left CST. There was no obvious bias in the lateralization of initial limb symptoms. Concordance has been observed for handedness and site of first symptoms in patients with upper limb–onset ALS,[39] but less than half of our patients (10 of 24) had right-sided limb onset of symptoms and fewer (7 of 24) had upper limb onset in a group of nearly all right-handed patients and control subjects. Other DTI studies in ALS have reported apparent lateralization of CST involvement to the right upper CST.[13,40] There is also an under-recognized post mortem observation of potential relevance that the human CST is asymmetric in 75% of individuals, with 75% of these cases having a larger decussation to the right-sided cord CST.[41]

However, FA changes have been observed in the PLIC on both affected and nonaffected sides according to disease burden,[32] and they appear bilaterally in DTI meta-analysis.[35] Correlations of CST FA were similar for both the left and right sides in our data. Therefore, we remain cautious in the interpretation of any apparent lateralization of PLIC (or wider CST) vulnerability but suggest that the specific longitudinal study of patients with ALS with strongly lateralized signs may be valuable in clarifying this issue.

FA AS A PROGNOSTIC VS MONITORING BIOMARKER

In most clinical studies in ALS, assessments will inevitably be made in groups of patients with variable progression slopes, disease duration, and disability at the time of study, as well as differing stages in terms of their total disease course (see Figure 1 in study by Grosskreutz et al[42]). For these reasons alone, it is perhaps not surprising that some DTI studies have failed to demonstrate significant reductions in FA longitudinally,[10] including this study. Potential issues of heterogeneity and method differences notwithstanding, 2 studies (1 from our group) have however specifically noted paradoxically greater FA values in those patients with longer disease duration (ie, slower progression rates).[6,9] We previously speculated that "the CST may be more resistant to disease-related damage in some patients with ALS, or such individuals have a higher baseline FA."[6] This study may lend further support to this concept (ie, FA may better reflect prognosis rather than longitudinal change).

The lack of correlation of any callosal DTI measures with progression rate may reflect the more consistent and perhaps very early involvement of this tract in ALS pathogenesis.

Post hoc, we considered the potential confounding effects of variable UMN involvement in the patients, using a pathologic reflex sum score gathered as part of the clinical assessment (see study by Filippini et al[6]). High UMN scores were significantly correlated with lower FA values in the CSTs (data not shown), in keeping with previous observations.[6,43] However, as a general observation, patients with UMN-predominant ALS and those with PLS (UMN only, by definition) are recognized to show consistently slower rates of progression.[44,45] Thus, UMN involvement potentially antagonizes the relationship between FA and progression rate. However, after adding the UMN score as an additional covariate of no interest, we still observed a significant negative correlation between CST FA and progression rate (data not shown), suggesting that FA is an independent prognostic factor.

As a wider point, we encourage reanalysis of imaging (and other biomarker) data sets using overall survival once this is known for the entire cohort. This will allow the disease stage at which investigation was performed to be used as a covariate, which may help to untangle prognostic hetero-

geneity when used in conjunction with other factors such as ALSFRS-R score, regional involvement, UMN vs lower motor neuron burden, and cognitive impairment.

CONCLUSION

Fractional anisotropy within the PLIC appears to have promising potential in identifying those cases of ALS associated with rapid progression. Further survival analyses are needed, ideally capturing the much more elusive short duration–slow progression patients. For the broader aim of the development of candidate neuroimaging surrogate markers that provide clear benefit over simpler and cheaper clinical measures such as the ALSFRS-R score, it seems likely that it will need to involve a multimodal approach with functional as well as structural measures,[46] possibly with additional cerebrospinal fluid markers.[47] The addition of a baseline and interval multimodal MRI to the protocol of future therapeutic trials seems a logical and imperative step toward this aim. This will require standardization and harmonization of MRI acquisition and analysis across international sites, an early framework for which has been established.[48]

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