Method supplement

Diffusion images were distortion, eddy current, and motion corrected,(Andersson and Sotiropoulos 2016) prior to calculating conventional DTI metrics (fractional anisotropy [FA], mean diffusivity [MD], axial diffusivity [AD], and radial diffusivity [RD]). Four regions were created in MNI standard coordinate space and then transformed to the DTI space of each subject using nearest-neighbour interpolation.(Desikan, Segonne et al. 2006, Avants, Duda et al. 2008, Li, Morgan et al. 2016) Specifically, a cubic region (volume $3\times3\times3$ mm^3) was centred in MNI space at coordinates [27, 52, 50], [32, 52, 50], [63, 52, 50], and [58, 52, 50] in order to locate left association, left projection, right association, and right projection fibers at the level of the lateral ventricle body. A representative image showing placement of the region of interest is included in Supplemental Figure 1. Values of the DTI metric from each region were obtained using *fslstats* within FSL.(Smith, Jenkinson et al. 2004) Because no laterality effects were detected a single bilateral DTI-ALPS was used in the analysis.

Statistical analysis of the DTI-ALPS data used R and RStudio, with packages ggplot2, lmerTest, ggsignif and AICcmodavg.(Wilkinson 2011, Kuznetsova, Brockhoff and Christensen 2017, Constantin 2021, Mazerolle 2023)

Patient vs control
The first model compared DTI-ALPS values in all patients vs all controls along with the impact of time, and the interaction between time and disorder status, controlling for age and sex with subject ID included as a random variable, using the following model:

$Y_{(DTI\text{-}ALPS)} = \beta_0 + \beta_1 Timepoint + \beta_2 Disease\ Status + \beta_3 Age + \beta_4 Sex + \beta_5 Timepoint*Disease\ Status + random(Subject\ ID) + \varepsilon$

Diagnosis Model
The above model was repeated, including specific diagnosis instead of disease status, so that ALS, PLS and Controls were examined separately.

$Y_{(DTI\text{-}ALPS)} = \beta_0 + \beta_1 Timepoint + \beta_2 Diagnosis + \beta_3 Age + \beta_4 Sex + \beta_5 Timepoint*Diagnosis + random(Subject\ ID) + \varepsilon$

Symptom Model
As an exploratory step, a third mixed-effects model including the patients only, was used to test the relationship between the DTI-ALPS index, the ALSFRS score, and the estimated progression rate and an interaction with timepoint for both variables.

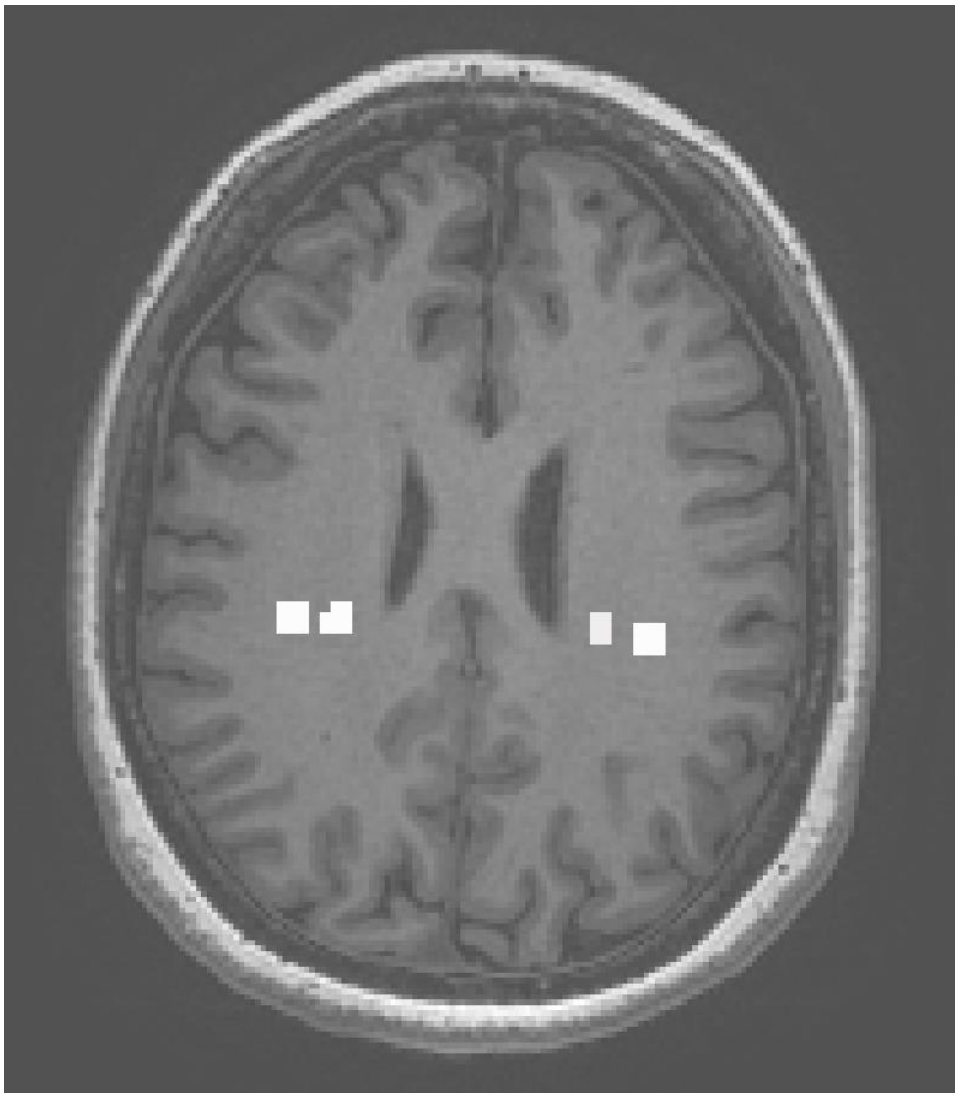
$Y_{(DTI\text{-}ALPS)} = \beta_0 + \beta_1 Timepoint + \beta_2 Diagnosis + \beta_3 Age + \beta_4 Sex + \beta_5 ALSFRS\ Total\ Score + \beta_6 Estimated\ Progression\ Rate + \beta_7 Diagnosis*Estimated\ Progression\ Rate + \beta_8 Diagnosis*ALSFRS\ Score + \beta_9 Timepoint*Estimated\ Progression\ Rate + \beta_{10} Timepoint*ALSFRS\ Score + random(Subject\ ID) + \varepsilon$

Fazekas Score
Both the Diagnosis Model and the Symptom model were repeated including the modified Fazekas score:

$Y_{(DTI\text{-}ALPS)} = \beta_0 + \beta_1 Timepoint + \beta_2 Diagnosis + \beta_3 Age + \beta_4 Sex + \beta_5 Fazekas\ Score + \beta_6 Timepoint*Diagnosis + random(Subject\ ID) + \varepsilon$

$Y_{(DTI\text{-}ALPS)} = \beta_0 + \beta_1 Timepoint + \beta_2 Diagnosis + \beta_3 Age + \beta_4 Sex + \beta_5 ALSFRS\ Total\ Score + \beta_6 Estimated\ Progression\ Rate + \beta_7 Fazekas\ Score + \beta_8 Diagnosis*Estimated\ Progression\ Rate + \beta_9 Diagnosis*ALSFRS\ Score + \beta_{10} Timepoint*Estimated\ Progression\ Rate + \beta_{11} Timepoint*ALSFRS\ Score + random(Subject\ ID) + \varepsilon$

Supplemental Figure 1



Representative image showing a transverse axial MRI image and placement of regions of interest for the DTI-ALPS analysis.

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