On-line Supplemental Material

MR Imaging Acquisition

MR imaging scans were obtained using a 1.5T Avanto system (Siemens, Erlangen, Germany). The following sequences were obtained: 1) dual-echo turbo spin-echo (repetition time [TR]=2650 ms, echo time [TE]=28/113 ms, echo train length=5, 50 axial sections, section thickness=2.5 mm, matrix size=512×512, field of view [FOV]=250×250 mm^2); 2) FLAIR (TR=10000 ms, TE=115 ms, inversion time=2500 ms, flip angle=150°, number of sections=50, section thickness=2.5 mm with no gap, matrix size=256x224, FOV=240x210 mm^2); and 3) pulsed-gradient spin-echo echo-planar (TR=6500 ms, TE=95 ms, 40 axial sections, section thickness=2.5 mm, matrix= 128x128, FOV=240 x 240 mm^2; diffusion-encoding gradients applied in 12 non collinear directions, b factor=1000 s/mm^2, number of averages=8).

MR Imaging Analysis

All MR imaging analysis was performed by a single experienced observer, blinded to clinical and cognitive findings. Corticospinal tract hyperintensities were identified on dual-echo and FLAIR scans.

DT analysis was carried out using an in-house software.[1] Diffusion weighted images were first corrected for distortion induced by eddy currents.[2] The DT was then estimated by linear regression and mean diffusivity (MD) and fractional anisotropy (FA) maps were computed.[3] In addition, axial diffusivity (axD - which is equivalent to the magnitude of the largest eigenvalue of the tensor) and radial diffusivity (radD - which is the average of the two smallest eigenvalues of the tensor) maps were calculated. In order to minimize cerebrospinal fluid (CSF) contamination secondary to brain atrophy, CSF was masked out from the diffusivities and anisotropy maps. CSF segmentation was conducted on DE images using the FMRIB Automated Segmentation Tool (FAST) implemented within FSL4 library (http://www.fmrib.ox.ac.uk/fsl).

An atlas-based automated approach was used to obtain DT MR imaging-derived metrics of WM tracts. This procedure involves: 1) the creation of a reference FA image in the standard space (the FA atlas) using a group of healthy subjects (reference group), 2) the definition of WM tract probability maps on the FA atlas, 3) the non-linear alignment of individual study subjects' MD, FA, axD and radD maps to the FA atlas, and (iv) the application of WM tract probability maps to the individual subject's images to measure mean tract MD, FA, axD, and radD. In detail, the analysis was performed as follows:

FA Atlas Creation. Since the direct application of tractography in patients is hampered by the presence of regions with decreased FA (e.g., T2 hyperintensities) and brain atrophy, the FA atlas was obtained using DT MR images from 24 healthy volunteers (reference group) aged between 20 and 45 years (mean age ± SD= 31±8 years; women: 15) with no history of neurological or psychiatric disorders, as previously described.[4] Briefly, DE scans of the reference group were registered to standard Montreal Neurological Institute space with affine transformation using the VTK CISG Registration Toolkit. This transformation was then applied to FA images to correct for differences in head size between controls. FA maps were then non-linearly transformed with an iterative procedure to produce an average shape and intensity image atlas (i.e., the FA atlas).[4]

WM Tract Probability Maps. On FA maps of reference healthy subjects, fiber tracking was performed to obtain the major cerebral WM tracts, bilaterally. These included the CST, corpus callosum, cingulum, superior longitudinal fasciculus, inferior fronto-occipital fasciculus, inferior longitudinal fasciculus, uncinate fasciculus, and fornix. WM tracts of the reference subjects were then registered to the standard space using the transformation matrices computed previously and averaged to produce WM tract probability maps. These maps were then thresholded at 40%. Finally, a skeleton (http://www.fmrib.ox.ac.uk/fsl/data/FMRIB58_FA.html) was applied to the tract probability maps in order to consider only the centre of each tract and thus minimize further any partial volume effect. Right and left measures for each tract were obtained.

WM Tract DT MR Imaging Metrics. The nonlinear transformations between the FA atlas and the FA maps of each study subject were estimated, and then applied to each subject's MD, FA, as well as axD and radD maps. WM tract probability maps were used as masks to obtain average MD, FA, axD and radD values of each WM tract.

References

1. Filippi M, Cercignani M, Inglese M, et al. **Diffusion tensor magnetic resonance imaging in multiple sclerosis.** *Neurology* 2001;56:304–311
2. Studholme C, Hill DL, Hawkes DJ. **Automated three-dimensional registration of magnetic resonance and positron emission tomography brain images by multiresolution optimization of voxel similarity measures.** *Med Phys* 1997;24:25–35
3. Basser PJ, Pierpaoli C. **Microstructural and physiological features of tissues elucidated by quantitative-diffusion-tensor MRI.** *J Magn Reson B* 1996; 111:209–219
4. Pagani E, Agosta F, Rocca MA, et al. **Voxel-based analysis derived from fractional anisotropy images of white matter volume changes with aging.** *NeuroImage* 2008;41:657–667