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Changes in white matter microstructure during adolescence

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Postmortem histological studies have demonstrated that myelination in human brain white matter (WM) continues throughout adolescence and well into adulthood. We used in vivo diffusion-weighted magnetic resonance imaging to test for age-related WM changes in 42 adolescents and 20 young adults. Tract-Based Spatial Statistics (TBSS) analysis of the adolescent data identified widespread age-related increases in fractional anisotropy (FA) that were most significant in clusters including the body of the corpus callosum and right superior corona radiata. These changes were driven by changes in perpendicular, rather than parallel, diffusivity. These WM clusters were used as seeds for probabilistic tractography, allowing us to identify the regions as belonging to callosal, corticospinal, and prefrontal tracts. We also performed voxel-based morphometry-style analysis of conventional T1-weighted images to test for age-related changes in grey matter (GM). We identified a cluster including right middle frontal and precentral gyri that showed an age-related decrease in GM density through adolescence and connected with the tracts showing age-related WM FA increases. The GM density decrease was highly significantly correlated with the WM FA increase in the connected cluster. Age-related changes in FA were much less prominent in the young adult group, but we did find a significant age-related increase in FA in the right superior longitudinal fascicle, suggesting that structural development of this pathway continues into adulthood. Our results suggest that significant microstructural changes in WM continue throughout adolescence and are associated with corresponding age-related changes in cortical GM regions.

Keywords: Diffusion MRI; Tractography; Adolescence

Introduction

The notion that myelination in brain white matter is not complete by childhood, but continues throughout adolescence and adulthood, has been demonstrated by both conventional structural magnetic resonance imaging (MRI) studies (Courchesne et al., 2000; Paus et al., 1999; Pfefferbaum et al., 1994) and postmortem histological analysis (Benes et al., 1994). In recent years, diffusion tensor magnetic resonance imaging (DTI) has been applied to address this issue, by providing sensitive measures of the changes in the microstructure of white matter (WM) in the brain that occur over childhood.

DTI is sensitive to the self-diffusion of water molecules. By fitting a model (such as the diffusion tensor model) to the diffusion measurements at each voxel, it is possible to estimate useful parameters such as the fractional anisotropy (FA) and the three principal diffusivities of the diffusion tensor (eigenvalues: λ_1, λ_2 and λ_3) (Basser, 1995). FA is a measure of the degree of diffusion directionality and ranges from 0 (isotropic diffusion) to 1 (anisotropic diffusion). Increasing FA values over development can indicate an increased compactness or density of the fibre bundles or an increased myelination (Beaulieu, 2002), although interpretation of changes in diffusion parameters is not always straightforward, and other factors such as tract geometry will influence FA. The eigenvalues of the diffusion tensor, parallel (λ_1) or perpendicular (λ_2 and λ_3) to white matter tracts, are also important indices useful for a better characterization of the changes in tissue microenvironment (Cader et al., 2007; Oh et al., 2004; Schonberg et al., 2006).

Volumetric MRI studies have established that there are gross morphological changes in both grey and white matter structure during childhood (Giedd et al., 1999; Reiss et al., 1996) and adolescence (Giedd et al., 1999), although specific findings are inconsistent. DTI studies in childhood have suggested that changes in white matter microstructure occur with development (Barnea-

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Goraly et al., 2005; Schmithorst et al., 2005; Snook et al., 2005), but there is not yet a consensus regarding the distribution and extent of these changes. More limited DTI data are available regarding age-related changes in brain white matter during adolescence and, again, specific findings are mixed. Positive correlations between age and FA have been reported in groups which extend from childhood to adolescence in the internal capsule, pyramidal tracts, left arcuate fascicle and right inferior longitudinal fascicle (Schmithorst et al., 2002), and also in the ventral visual pathways, basal ganglia, thalamic pathways and corpus callosum (Barnea-Goraly et al., 2005). Investigation of the time course of these changes suggests that in general FA increases are steepest before the age of 10 and begin to plateau in later childhood and adolescence (Ben Bashat et al., 2005). Certain structures, however, such as the centrum semiovale and splenium of the corpus callosum show strongest FA changes in adolescence (Ben Bashat et al., 2005).

Although numerous studies have reported decline in white matter FA with ageing beyond the age of 50 (Abe et al., 2002; Bhagat and Beaulieu, 2004; Moseley, 2002; Nusbaum et al., 2001; Pfefferbaum et al., 2000; Salat et al., 2005), few studies have investigated age-related white matter changes in younger adults. Age-related changes in young adulthood are much less pronounced than those observed in children and elderly adults. One study found a global increase in mean FA based on histogram analyses of white matter in subjects aged 20 to 40 (Yoshiura et al., 2005), while a localized increase in FA in the right centrum semiovale has been reported for subjects between the ages of 21 and 27 (Snook et al., 2005). Increasing FA in the right centrum semiovale was associated with a decline in perpendicular diffusivities, rather than an increase in the parallel diffusivity (Snook et al., 2005), consistent with the hypothesis that increasing fibre compactness and/or myelination drives the FA increase.

In summary, although age-related changes in white matter microstructure have been much studied in early childhood and late adulthood, changes occurring in adolescence and early adulthood are less well characterized. In the present study, we sought to define age-related structural white matter changes in adolescents (13.5–21 years) and young adults (23–42 years) using a novel DTI approach to test for voxelwise correlations between diffusion parameters and age within each group.

Methods

Data acquisition

We acquired MR data in two groups: a group of forty-two healthy adolescent subjects (22 males, 20 females; age range 13.5–21 years; 40 right-handed, 2 left-handed) and a group of twenty adult subjects (11 males, 9 females; age range 23–42 years; all right-handed). The age chosen to differentiate between adolescence and young adulthood varies by a few years between studies, but is typically between 17 and 22 years; our choice of 21 years is broadly consistent with previous work (Giedd et al., 1999; Snook et al., 2005). None of the subjects had a history of psychiatric or neurological disease. Informed written consent was obtained from all subjects in accordance with ethical approvals from the Central Office for Research Ethics Committees (04/Q1607/64, 05/Q1606/8, C002.064).

Scans were obtained on a 1.5 T Siemens Sonata MR scanner with maximum gradient strength of 40 mT m^{-1}. Diffusion-weighted data were acquired using echo planar imaging (TR = 8500 ms; TE = 80 ms; 53 × 2.5 mm thick axial slices; voxel size of 2.5 × 2.5 × 2.5 mm^3). The diffusion weighting was isotropically distributed in 60 directions using a b value of 1000 s mm^{-2}. A T1-weighted image was also acquired for each subject for a voxel-based morphometry-style (VBM) analysis and image registration (3D FLASH; TR = 12 ms: TE = 5.6 ms; 1 mm isotropic voxels; matrix = 256 × 256 × 208; NEX = 3 Elliptical sampling; orientation = coronal).

To test our DTI analysis approach on data showing a robust age effect with other techniques, we also separately analysed a pediatric data set (n = 9, age range 0–56 months) that has been generously made publicly accessible (www.pediatricDTI.org), and forms part of a larger database previously used to demonstrate age-related effects (Hermoye et al., 2006). Note that these data were acquired using different DWI parameters to those used to collect the adolescent and adult data presented here. However, as no comparisons are made between the pediatric data and the adolescent and adult data this was not thought to raise any problems.

Data analysis

Diffusion data analysis

FMRIB's Diffusion Toolbox (FDT), part of the FMRIB Software Library (FSL, www.fmrib.ox.ac.uk/fsl), was used to fit a diffusion tensor model to the data at each voxel. Voxelwise values of fractional anisotropy (FA) and diffusivity parallel (λ_1) and perpendicular ($(\lambda_2+\lambda_3)/2$) to the principal diffusion direction were calculated.

We then used Tract-Based Spatial Statistics (TBSS, also part of FSL), a method for voxelwise statistical comparison of diffusion indices between individuals (Smith et al., 2006), to test for local correlations between age and FA across the whole brain white matter. First, individual FA maps were non-linearly aligned using spline-based free-form deformation (Rueckert et al., 1999) (implemented in the Image Registration Toolkit, www.doc.ic.ac.uk/~dr). The cross-subject mean FA image was calculated and used to generate a white matter tract 'skeleton', which was thresholded at FA > 0.2. The resulting skeleton included 157,263 1 × 1 × 1 mm^3 white matter voxels (corresponding to approximately half of the WM voxels with FA > 0.2). Individual subject FA values were warped onto this group skeleton for statistical comparisons by searching perpendicular from the skeleton for maximum FA values. Maximum FA values are chosen in order to restrict analysis to the centres of WM tracts (where maximum FA values will be found), rather than considering voxels on the edges of tracts, that may suffer from partial volume effects. Values from these same voxels for the parallel and perpendicular components of the diffusion tensor were also mapped onto the skeleton and compared statistically.

To aid comparison with previous studies that have used conventional voxelwise analysis of FA values, we also tested for correlation between age and FA across all white matter values (FA > 0.2), after non-linear registration, but before remapping onto the TBSS skeleton.

To test for global changes in diffusion measures we calculated mean FA and diffusivity values both across the whole white matter skeleton and across the entire brain white matter for both subject groups and performed within-group correlations with age using Pearson's correlation coefficient.

To test for local correlations between age and diffusion measures, we used the randomise program within FSL to carry out permutation-based testing (Nichols and Holmes, 2002). Inference was carried out using cluster-size thresholding. Clusters are defined by first thresholding the raw *t*-statistics map on the skeleton at $t>3$, and finding contiguous clusters of supra-threshold voxels, using 26-neighbour connectivity. The null distribution of the cluster-size statistic was built up over 5000 random permutations of group membership, with the maximum size (across space) recorded at each permutation. The 95th percentile of this distribution (a cluster size of approximately 150 voxels on the skeleton) was then used as the cluster-size threshold, i.e., the clusters were thresholded at a level of $p<0.05$, which is fully corrected for multiple comparisons across space (i.e., controlling the familywise error – the chance of one or more false positives anywhere on the skeleton).

Within clusters showing significant age-related correlations with TBSS, we calculated the mean FA across all voxels within the cluster in each subject and plotted this against age to visualize the spread of values across the group. Values for mean FA and eigenvalues were correlated with age in both groups using Pearson's correlation coefficient. *P* values of <0.05 (uncorrected for multiple comparisons) were considered significant.

Clusters showing significant age-related correlation with TBSS were also used as seed masks for probabilistic tractography (Behrens et al., 2003). Briefly, at each voxel, we estimated a probability density function (PDF) on the principal fibre direction. Tractography then proceeded by drawing multiple (in this case 5000) streamline samples through these PDFs from each seed voxel to build up an estimate of the distribution of connections from each seed voxel. Generated pathways are volumes in which values at each voxel represent the number of samples passing through that voxel and, therefore, the probability of connection to the seed voxel. To remove spurious connections, pathways in individual subjects were thresholded to include only voxels in which at least 20 samples (out of 5000 generated from each seed voxel) passed through. Pathways in each subject were then binarised and overlaid to produce population probability maps for each pathway in which voxel values reflect the proportion of subjects in whom a pathway is present.

Grey matter voxel-based morphometry-style analysis

To assess differences in the topographic distribution of grey matter (GM) in adolescents, an optimised VBM-style analysis (Good et al., 2001) using FSL tools for brain extraction and segmentation and the same non-linear registration that was used for the TBSS analysis (Rueckert et al., 1999) was carried out. First, tissue type segmentation was used to generate partial volume estimates for each subject based on native T1-weighted images. Then, a symmetric study-specific GM template was created by averaging the 42 GM-segmented native images and their respective mirror images that were all affine registered to the ICBM 152 GM template. The GM native images were then non-linearly transformed to the study-specific template. The optimised protocol includes a compensation (or "modulation") for the contraction/enlargement of brain areas due to the non-linear component of the transformation. This entails multiplying the values in each voxel of a registered image by the inverse of the warp field's Jacobian. Finally, the modulated GM images were smoothed with an isotropic Gaussian kernel with a sigma of 3.5 mm (~8 mm FWHM). Resulting images of 'GM density' therefore represent the partial volume estimate of GM per voxel (ranging between 0 and 1), averaged over the size of the smoothing kernel. Statistical analysis of modulated GM values was then performed using permutation testing (Nichols and Holmes, 2002) using the randomise program in FSL as for the diffusion analysis.

Results

Adolescent group: Within-group correlations between age and diffusion measures

To test for global age-related change in diffusion measures, we tested for correlations between age and diffusion measures averaged across the whole white matter skeleton. Within the adolescent group, we found a significant positive correlation between age and mean FA across the skeleton ($r=0.42$, $p<0.01$) (Fig. 1A). This correlation remained significant if FA across all WM voxels was considered (rather than only those voxels within the skeleton) ($r=0.41$, $p<0.01$).

Increases in FA with age could be driven by increases in the diffusion parallel to the principal diffusion direction or decreases in diffusion perpendicular to the principal diffusion direction. To test the relative contribution of parallel and perpendicular diffusivity to this age-related change, we also separately tested correlations between age and parallel or perpendicular diffusivity across the whole white matter skeleton. Parallel diffusivity did not correlate with age (Fig. 1B), while perpendicular diffusivity decreased significantly with increasing age ($r=-0.35$, $p<0.01$) (Fig. 1C), suggesting that the observed FA change is primarily driven by decreases in diffusion perpendicular to the principal diffusion direction.

We then carried out stringent statistical thresholding within TBSS to identify only those clusters that survived multiple comparison correction in order to identify the regions showing the strongest age-related change in FA. Using cluster-based thresholding, we found the strongest positive correlations between FA and age within two white matter regions (Table 1; Figs. 2A and C). These clusters were used as seeds for probabilistic tractography, which allowed us to identify these regions as belonging to the right part of the body of corpus callosum (CC) and to the right superior region of the corona radiata (SCR). The paths seeded from the right part of the body of CC projected to the medial motor areas (Fig. 2B) and the cingulate gyrus whereas the paths seeded from the right SCR projected along the corticospinal tract and also branched anteriorly into the right middle frontal gyrus (Figs. 2D, H–J). Within the two clusters, correlation between mean FA and age was $r=0.55$ and $r=0.56$, respectively ($p<0.001$, Figs. 2E and F). Conventional voxelwise analysis of FA values identified a single cluster, also in the corona radiata (901 voxels; centre of gravity: 25, −4, 39), close to the cluster identified by TBSS.

We also carried out TBSS analysis using voxelwise values of parallel or perpendicular diffusivity. After correcting for multiple comparisons, no clusters showed a correlation between age and parallel diffusivity. However, a significant negative correlation with age was found for perpendicular diffusivity in a white matter cluster in the body of corpus callosum, overlapping with the cluster derived from cluster-corrected TBSS of FA ($r=-0.53$, $p<0.001$) (Fig. 2G).

To test for age-related effects on FA that are not well captured by a linear regression analysis including all subjects, we also

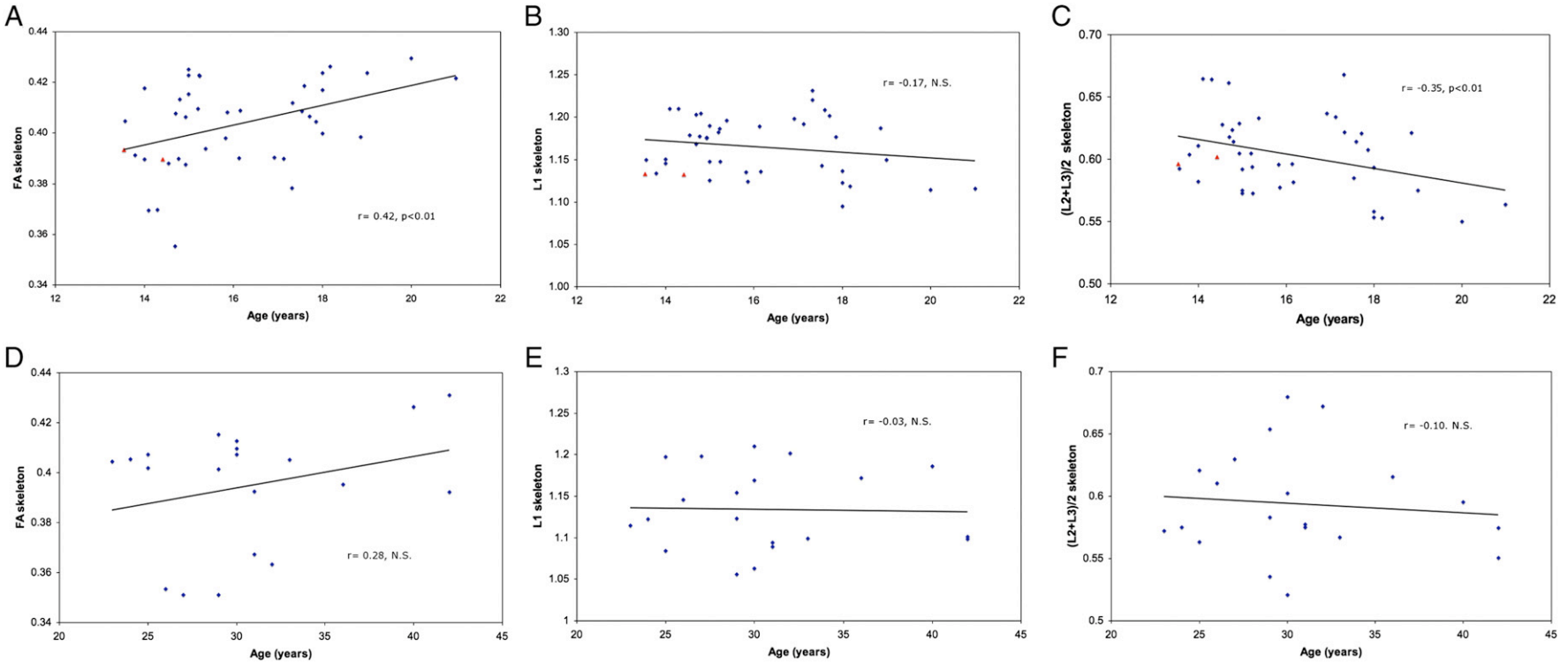


Fig. 1. Scatterplots showing correlations between age and diffusion parameters for the adolescents (top row) and young adults (bottom row). Correlations are shown between age and fractional anisotropy (A, D), parallel (B, E) and perpendicular (C, F) diffusivity. L1 represents the parallel diffusivity; (L2+L3)/2 represents the perpendicular diffusivity. The red triangles on the top row represent the two left-handed adolescents.

Table 1
Details of clusters showing a significant relationship between age and FA

Group	Contrast	Cluster location	Cluster size in voxels	Centre of gravity, *X*, *Y*, *Z*
Adolescents	Linear correlation with age	Right body of corpus callosum	212	11, −4, 33
	Linear correlation with age	Right superior region of corona radiata	145	25, −6, 38
	15 oldest vs. 15 youngest	Right body of corpus callosum	208	10, −3, 32
	15 oldest vs. 15 youngest	Left body of corpus callosum	263	−10, −8, 32
Adults	Linear correlation with age	Right superior longitudinal fascicle	182	37, −39, 35

compared FA values in a subset of the 15 youngest subjects (5 males, 10 females; age range 13.5–15 years; 13 right-handed, 2 left-handed), to the 15 oldest ones (13 males, 2 females; age range 17–21 years). The only white matter region surviving cluster-based thresholding was the body of CC, where we found two clusters, one on either side of the midline, showing age-related FA change (Table 1).

We also tested for FA differences due to gender and interactions between gender and age in the adolescents but did not find any significant effects.

Adolescent group: Within-group correlations between age and grey matter density measures

We did not find significant linear correlations between voxelwise GM values and age in the adolescent group. We also tested for significant age-related GM changes by dividing the adolescents into two subgroups containing the 15 youngest and the 15 oldest subjects as for the FA analysis. We found a trend for increased GM density in the youngest subgroup in the right middle frontal gyrus and in the right precentral gyrus after correction for multiple comparisons ($t>2.5$, $p<0.10$). This GM region includes the middle frontal gyrus location to which the WM tracts showing age-related FA changes projected (Figs. 2H–J). This cluster was used as a region of interest (ROI) to extract individual subject mean GM density values which allowed us to demonstrate a statistically significant relationship between decreased GM density in the cortex and increased FA in the WM SCR cluster identified by TBSS and located in the connecting tracts (Fig. 2K, $r=-0.65$, $p<0.001$). As the subgroups of oldest and youngest subjects used to define our GM cluster were not gender-matched, we also tested the partial correlation between FA and GM density, controlling for gender and found that the correlation remained highly significant ($r=-0.62$, $p<0.001$).

Young adult group: Within-group correlations between age and diffusion measures

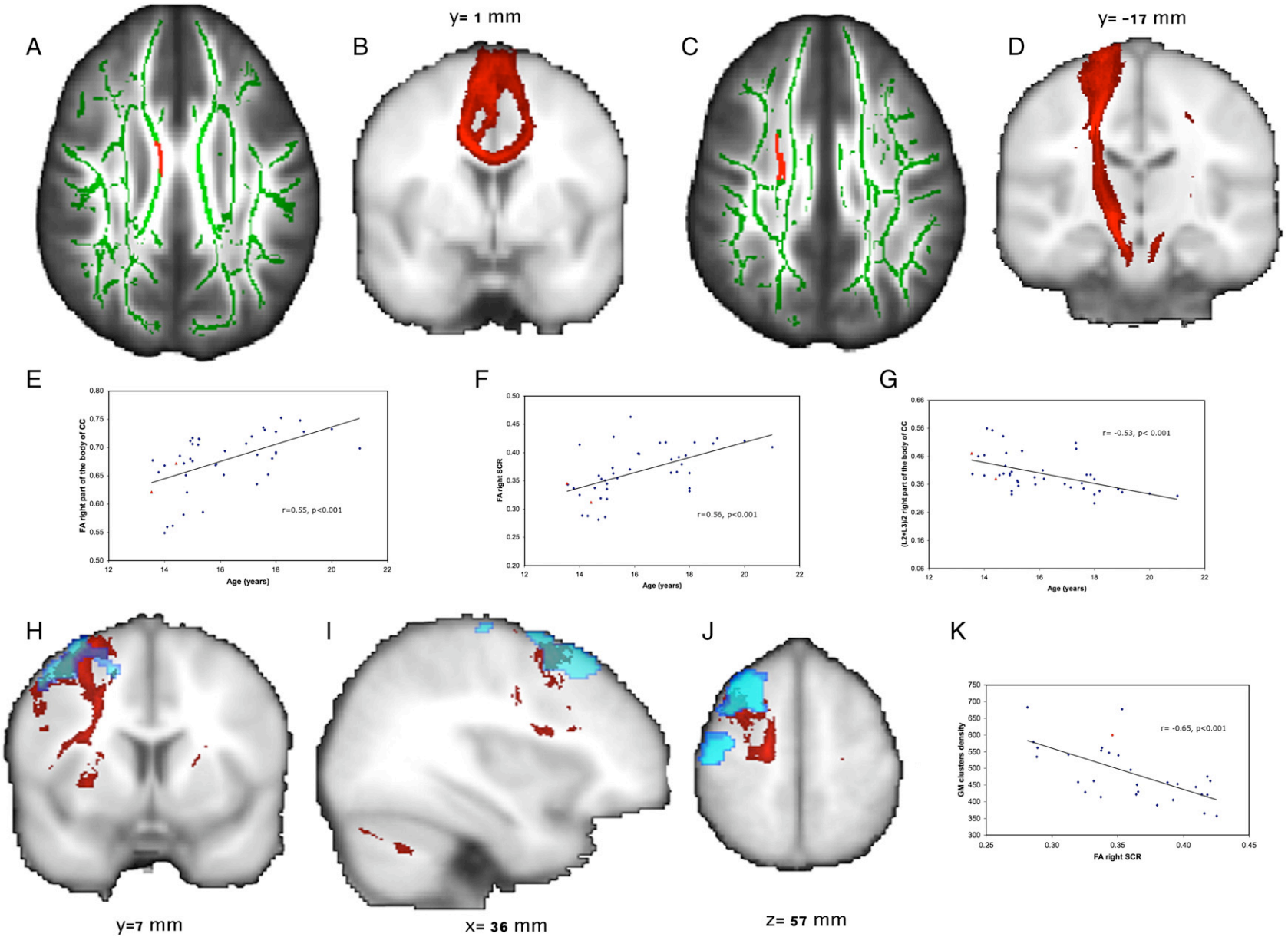
In contrast to the adolescent group, the young adult group did not show a significant correlation between age and mean FA ($r=0.28$, n.s.) (Fig. 1D) or mean diffusion tensor eigenvalues ($r=-0.03$, n.s. for parallel diffusivity; $r=-0.10$, n.s. for perpendicular diffusivity) (Figs. 1E and F) averaged across the white matter skeleton. Testing for correlations across the whole brain white matter also failed to show any significant results for this group.

When we tested for local correlations between age and FA using TBSS, the only region that survived cluster-based correction for multiple comparisons was within the right superior longitudinal fascicle (SLF), where we found a positive correlation between FA and age (Table 1; Figs. 3A and B). An ROI analysis of mean FA values within this cluster showed a correlation between FA and age of $r=0.67$ ($p<0.001$) (Fig. 3C). No voxels showed significant correlation between age and parallel or perpendicular diffusivity. No voxels showed a significant correlation between age and FA using a conventional voxelwise analysis.

TBSS on a previously studied pediatric population

To further validate the TBSS approach using data previously demonstrated to show a robust age effect, we performed TBSS analysis on a sample of a pediatric data set in which significant age-related changes in FA have been found using a conventional ROI-based approach (Hermoye et al., 2006). We compared changes in the white matter skeleton that we have defined here with those of the nine manually defined WM ROIs that the previous study had defined as showing age-related change. Using TBSS, we also found significant correlations between FA and age ($t>3$, $p<0.05$) bilaterally in the SCR, anterior region of corona radiata (ACR), posterior region of corona radiata (PCR), SLF, frontal U-fibres, anterior thalamic radiations (ATRs), forceps minor, anterior limb of the internal capsule (ALIC), external capsule (EC), inferior fronto-occipital fascicle/inferior longitudinal fascicle (IFO/ILF) and posterior limb of the internal capsule (PLIC). A few white matter regions (splenium of CC, genu of CC, cingulum (Cg) and fornix (Fx)) that were demonstrated to show age-related effects in the previous study did not survive cluster-based thresholding using TBSS with a threshold of $t>3$. However, after lowering our height threshold to $t>2$, we also found age correlations in the genu of CC and left Cg that survived cluster-based correction for multiple

Fig. 2. Local correlations between FA and age in the adolescent group. TBSS analysis identified significant positive correlations (indicated by red voxels) between age and FA in the right body of corpus callosum (A) and right superior corona radiata (C) (cluster-based correction for multiple comparisons ($t>3$, $p<0.05$)), green voxels indicate the white matter skeleton and the background image is the mean FA image. In panels B and D, probabilistic tractography is used to trace pathways from the clusters shown in panels A and C, respectively. Panels E–G show scatterplots of correlations between age and diffusion measures in clusters identified in panels A and C, respectively. Positive correlations are shown between FA and age for the right body of the CC (E) and right SCR (F) and negative correlation between age and perpendicular diffusivity ((L2+L3)/2) is shown for the right part of the body of CC (G). Panels H–J show the relationship between tracts from WM clusters showing age-related FA increase (in red, tracts from SCR cluster also shown in panel D), and the cortical region showing age-related GM decrease (in blue). GM density is expressed in mm^3.


A
B
y= 1 mm
C
D
y= -17 mm
E
FA right part of the body of CC
Age (years)
r=0.55, p<0.001
F
FA right SCR
Age (years)
r=0.56, p<0.001
G
(L2+L3)/2 right part of the body of CC
Age (years)
r= -0.53, p< 0.001
H
y=7 mm
I
x= 36 mm
J
z= 57 mm
K
GM clusters density
FA right SCR
r= -0.65, p<0.001

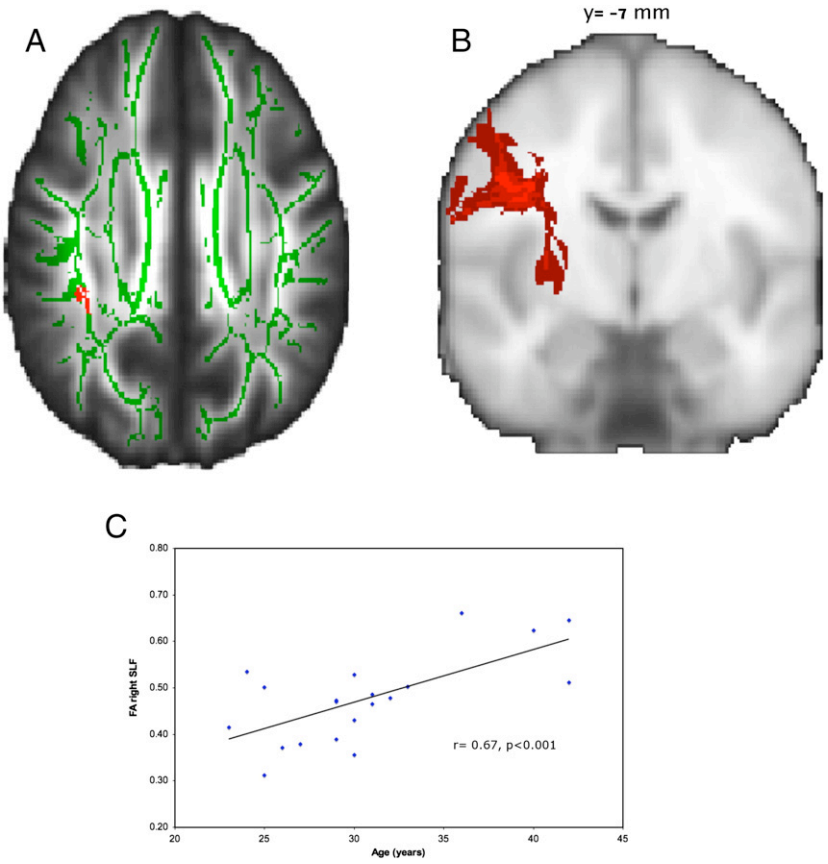


Fig. 3. Local correlations between FA and age in the young adult group. TBSS analysis identified a significant positive correlation (indicated by red voxels) between age and FA in the right SLF (A) (cluster-based correction for multiple comparisons ($t>3$, $p<0.05$)), green voxels indicate the white matter skeleton and the background image is the mean FA image. In panel B, probabilistic tractography is used to trace pathways from the cluster shown in panel A. Panel C shows the scatterplot of FA values versus age within the cluster shown in panel A.

comparisons ($p<0.05$). Age correlations were revealed in the splenium of CC, Fx and right Cg when the height threshold for cluster-based thresholding was lowered to $t>1$.

Discussion

We found widespread age-related increases in whole brain white matter FA in a group of adolescents (13.5–21 years). Stringent testing for local correlations between age and FA revealed the most significant changes to be in the right body of corpus callosum and in the right superior region of corona radiata. In a group of young adults (23–42 years), age-related changes in FA were less pronounced: we did not find evidence for global changes in FA, but a local positive correlation between age and FA within the right superior longitudinal fascicle was demonstrated.

These findings are in agreement with previous reports that age-related FA changes continue beyond early childhood (Ashtari et al., 2007; Barnea-Goraly et al., 2005; Bonekamp et al., 2007; Klingberg et al., 1999; Schmithorst et al., 2002). Our results suggest that these effects are widespread, as a positive relationship between FA and age is seen throughout the brain white matter, consistent with previous reports of global increases in white matter density using conventional MRI (Giedd et al., 1999). However, our findings also suggest that different white matter pathways mature at different rates: the steepest relationships between age and FA during adolescence were observed in the right body of the corpus callosum and superior region of the right corona radiata.

Our voxelwise findings of localized changes in the corpus callosum are consistent with previous reports using ROI-based analysis showing that the body of the corpus callosum undergoes structural maturation into late adolescence (Barnea-Goraly et al., 2005). The body of the corpus callosum contains fibres important for connecting motor and sensory cortices and an increase of FA at this location may be linked to an improvement of motor skills during development (Barnea-Goraly et al., 2005). When controlling for age, FA within this same region of the corpus callosum in healthy adult subjects correlates with bimanual motor skill ability (Johansen-Berg et al., 2007), suggesting that white matter microstructure may by associated with acquisition of age- or experience-related motor skills.

The second region showing highly significant age-related increases in FA during adolescence was the right corona radiata, the radiating bundle of fibres projecting to and from the whole cerebral cortex. Tractography from this region generated ascending paths to the motor cortices, in particular to the premotor and prefrontal areas, and descending paths through the internal capsule, suggesting that motor pathways such as the corticospinal tract are also displaying this age-related change. Previous reports have demonstrated that a similar region of central, subcortical white matter shows age-related increases in FA through adolescence and

even into early adulthood (Ben Bashat et al., 2005; Snook et al., 2005) and have reported increased FA (Ashtari et al., 2007; Bonekamp et al., 2007) or WM density (Paus et al., 1999) over adolescence in motor pathways at the level of the internal capsule. This latter study also found significant effects in the posterior portion of the left arcuate fascicle in a large group of children and adolescents (Paus et al., 1999), consistent with development of speech and language abilities over this time. Our failure to identify this pathway as showing age-related FA changes after stringent statistical correction most likely reflects the lower power of our study. In addition, there may be different sensitivities of FA versus WM density analyses to different aspects of WM change although other studies of FA changes have detected age-related increases in the left arcuate over late adolescence (Ashtari et al., 2007).

To test whether age-related changes in white matter microstructure were related to changes in interconnected regions of grey matter, we also performed voxel-based morphometry-style analysis of T1-weighted structural MR images. In contrast to our analysis of white matter FA, we did not find significant linear correlations between grey matter volume and age in the adolescent group, suggesting that white matter changes may be more prominent. We did, however, detect a trend towards decreased grey matter density in the right middle frontal gyrus and right precentral gyrus in the 15 oldest adolescents compared to the youngest adolescents. A decrease in GM from adolescence to young adulthood is consistent with the notion that cortical grey matter density shows regionally specific pre-adolescent increases followed by a post-adolescent decrease (Giedd et al., 1999). We had insufficient age range and power to test for such a non-linear pattern of developmental change in the current study, but our results in adolescence suggest that we may be picking up the later decline in GM density observed in previous studies that have tested a wider age range (Giedd et al., 1999). The cortical regions that we find to show decreasing GM were connected to the pathways generated from the right corona radiata region showing significant age-related FA increase. We used the GM region to define an ROI from which we extracted individual subject GM density values. We found a highly significant correlation between the increase in FA in the SCR WM and the decrease in GM density in the cortical ROI, suggesting that associated age-related changes may be occurring in both grey and white matter. Decreases in GM density inferred from imaging are ambiguous and could reflect either increased cortical myelination or decreased synaptic or cellular density (Paus, 2005). On the one hand, our finding that the age-related decrease in GM correlates with increases in WM FA is consistent with the hypothesis that both are driven by increased myelination. On the other hand, the localization of decreased GM density in prefrontal cortex is consistent with previous reports that synaptic pruning in this region continues into middle adolescence (Huttenlocher and Dabholkar, 1997).

To validate our DTI analysis approach on a data set known to contain significant age-related effects, we used TBSS to study a subset of a population of infants and very young children that were previously studied with a conventional ROI-based approach (Hermoye et al., 2006). In agreement with the previous study, our voxel-based approach found significant FA increases in the posterior limb of internal capsule, inferior fronto-occipital fascicle, superior longitudinal fascicle and frontal U-fibres. After lowering our statistical height threshold, we also detected age-related changes, corrected for multiple comparisons, in corpus callosum, cingulum and fornix, in agreement with the previous study. Moreover, because our analysis extended to the whole white matter skeleton, we found additional regions of correlation between age and FA, including the superior region of corona radiata, anterior region of corona radiata, anterior thalamic radiations, forceps minor, external capsule, posterior region of corona radiata; all of these changes were seen bilaterally. The age range taken into account was the same as the previous study (0–56 months), even though the number of subjects studied was limited to the subset of nine out of the original 30 subjects whose FA maps were available.

Our study benefited from some methodological advances compared to previous DTI investigations of age-related changes in FA. Specifically, we acquired data using 60 diffusion encoding directions providing increased power and higher angular resolution than previous investigations. For data analysis, we used tract-based spatial statistics (Smith et al., 2006), a novel technique for voxelwise analysis of diffusion parameters that alleviates some of the problems associated with conventional voxelwise approaches such as voxel-based morphometry. Finally, clusters that we found to have significant correlation between FA and age were used as seeds for probabilistic tractography (Behrens et al., 2003), allowing us to be more certain in the localization of our white matter changes to specific fibre pathways.

The mechanisms responsible for the age-related increases in FA remain to be elucidated. FA is a complex measure that will be influenced not only by myelination, axon size, and axon density (Beaulieu, 2002), but also by path geometry and the presence of crossing fibre pathways. It is well established that myelination continues into adolescence (Benes, 1989) and even into adulthood (Benes et al., 1994). This fits with the idea that skill learning and experience, which clearly continue throughout life, are accompanied by structural changes including myelination and sprouting (Beaulieu and Colonnier, 1987; Ichikawa et al., 1993; Turner and Greenough, 1985). Recent studies suggest that such behaviourally relevant variation in white matter microstructure may be reflected by changes in diffusion measures (Bengtsson et al., 2005; Madden et al., 2004; Tuch et al., 2005). As ageing progresses, age-related maturational changes and ageing-related degeneration (Kochunov et al., in press; O'Sullivan et al., 2001; Pfefferbaum et al., 2000) may act in an opposing fashion as part of the continued dynamics of myelin remodeling.

In addition to testing for correlations between age and FA, we also separately tested for correlations with the different eigenvalues of the diffusion tensor – representing diffusivity parallel or perpendicular to the principal diffusion direction. It has been argued that separate consideration of these components could shed light on potential mechanisms underlying changes in FA with disease, for example (Oh et al., 2004; Pierpaoli et al., 2001). Within-group age correlations showed a negative correlation between perpendicular diffusivity and age for the adolescent group only, suggesting that FA increases are driven primarily by decreases in perpendicular diffusivity. Such decreases are suggestive of an increase in the compactness or density of the fibre bundles and/or increased myelination (Snook et al., 2005) or a decline of unrestricted water content in extra-axonal space (Suzuki et al., 2003). Our findings are consistent with previous developmental DTI studies that have considered the contribution of the three principal eigenvalues on FA (Bhagat and Beaulieu, 2004; Snook et al., 2005; Suzuki et al., 2003), but contrary to one recent study of late adolescence that suggests that increases in parallel diffusivity drive age-related FA increases (Ashtari et al., 2007). Future studies should test whether factors such as variation

in data acquisition or subject characteristics could explain this discrepancy.

In summary, we have used a novel DTI analysis approach to study age-related changes in late adolescence and early adulthood. Our results suggest that developmental changes in white matter microstructure continue throughout adolescence but are particularly prominent in specific fibre pathways including the body of the corpus callosum, and descending motor pathways. Most white matter changes plateau in early adulthood, but properties of the cortical projection pathways travelling through the superior longitudinal fascicle appear to continue to change even into early adulthood.

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