POSSIBLE CONFOUNDING FACTORS ON CEREBRAL DIFFUSION TENSOR IMAGING MEASUREMENTS

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Avainsanat: aivot, diffuusiotensorikuvaus, ikä, tupakointi, sukupuoli, kätisyys, koulutusvuodet, alkoholinkäyttö

Tutkimuksen tarkoituksena oli tutkia iän, tupakoinnin, koulutusvuosien, sukupuolen, kätisyyden ja alkoholinkäytön vaikutusta terveiden henkilöiden aivojen diffuusiotensorikuvauksen tuloksiin eri aivoalueilla.

Tutkimuspopulaatio koostui 40 potilaasta, joille tehtiin aivojen diffuusiotensorikuvaus 3 Teslan laitteella. Diffuusiotensorikuvauksen muuttujina käytettiin FA- ja ADC-arvoja. ROI-menetelmää hyödyntäen määritettiin yhteensä 13 mittauskohtaa molempien aivopuoliskojen sekä corpus callosumin alueelle.

Tärkein löydös oli, että ikääntyminen vaikutti laskevasti FA-arvoihin etuaivojen alueella. Lisäksi tupakointi laski ADC-arvoja kolmella eri aivoalueella. Muiden tutkittavien tekijöiden suhteen tulokset olivat ristiriitaisia.

Tutkimuksen perusteella ikääntymistä ja tupakointia tulee pitää potentiaalisina sekoittavina tekijöinä ROI-menetelmään perustuvien diffuusiotensorikuvaustuloksien analysoinnissa. Sukupuolen, kätisyyden, alkoholinkäytön ja koulutusvuosien mahdolliset vaikutukset vaativat lisätutkimuksia.

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Abstract

Purpose: To investigate the possible confounding effects of age, gender, handedness, smoking, alcohol consumption and education on cerebral diffusion tensor imaging (DTI) parameters in a generally healthy homogenous sample with no neurological or psychiatric diseases.

Methods: Forty (n=40) subjects (mean age 40.3 years, SD 12.3) underwent DTI of the brain with 3T MRI. At enrolment, all the subjects were interviewed with respect to general health, handedness, education, history of smoking and alcohol consumption. Studied DTI parameters included: (i) fractional anisotropy (FA) and (ii) apparent diffusion coefficient (ADC). Region-of-interest (ROI) -based measurements were estimated at 13 anatomical locations bilaterally, except for the corpus callosum in which the ROIs were placed on the sagittal images. Circular-ROI measurements were mainly used. Freehand-ROI method was used with the forceps minor, uncinate fasciculus and thalamus. Intra-observer variability and repeatability were assessed for FA and ADC values.

Results: The most consistent finding was that aging decreased FA values in the frontal brain regions. This was apparent in four ROIs. Also, smoking decreased ADC values in three regions. However, the regions which were smoking-related had poor intra-observer repeatability. Regarding the other confounding factors, the results were discontinuous and no concrete conclusions could be drawn from these findings. In general, intra-observer repeatability of the DTI measurement was considered relatively good.

Conclusions: Age and smoking should be noted as considerable confounding factors in ROI-based DTI-analysis. More research on the effects of gender, handedness, alcohol consumption and education is needed.

Introduction

Diffusion tensor imaging (DTI) is a magnetic resonance imaging (MRI) technique which is used to visualized neural white matter tracts. DTI is based on the measures of the random diffusion motion of water in living tissues. Because of restricting structures such as cell membranes and myelin, the motion of water molecules is anisotropic (=directionally dependent) within neural fibres. The most cited quantitative parameters used to describe isotropy are: (i) fractional anisotropy (FA) and (ii) the apparent diffusion coefficient (ADC). FA measures the density of white matter and the degree of diffusion can be illustrated by ADC. The most commonly used methods for DTI analyses are region-of-interest (ROI) analysis and voxel-based analysis (VBA). Besides these, quantitative diffusion tractography, an alternative technique to ROI-measurements, can be used to characterize white matter tracts (1-4). ROI analysis is observer-dependent and time-consuming, whereas VBA is a fully automated and objective method. However, inter-subject registration and image smoothing are needed for using VBA. ROI-based method has been available for a longer time and it is therefore used in many previous DTI studies, being still the mostly used method in DTI analyses (5). The advantages of ROI analysis compared to VBA are: (i) the manual selection of regions, (ii) the quantification of the measured parameters and (iii) the individual applicability.

DTI techniques have been applied to numerous neurological and psychiatric conditions including stroke, multiple sclerosis (MS), schizophrenia, various dementias, traumatic brain injury, autism and depression. Commonly, increased FA and reduced ADC values relate to the initial phase of stroke (6). MS lesions can observe with increased ADC and decreased FA values. MS causes also fiber tract loss assessable with tractography (7-9). In neurodegenerative diseases, promising results have been achieved when DTI has been used in the early diagnosis of Alzheimer's disease. Studies have found increased ADC in most lobar regions and decreased FA values, particularly in sub-regions of the medial temporal lobes which are linked to cognitive impairment (3,10). By using DTI, neurodegenerative changes can also be found in patients with Parkinson's disease, specifically

reduced FA values in the substantia nigra (11). Additionally, DTI has been used to explore the damage of white matter tracts in patients with traumatic brain injury (12). In schizophrenic patients white matter disruptions have been found with DTI in several studies (13-15).

Reduced FA values and increased ADC values are related to a damage of the brain microstructure. Lower FA values can be attributed to a loss of axons or a damage of myelin sheaths. ADC increases when overall diffusion increases. ADC values can also rise during an inflammation and swelling. Generally, high FA values are indicators for high white matter "quality" (3).

In this study we examined how general systemic and environmental factors affect the brain microstructure of healthy adults. Several studies have shown age-related alterations in the structure of the human brain. According to these studies, lower FA and higher ADC values are associated with healthy aging (16-19). It is proven in many studies that men and women have dissimilarities in their brain microstructure (16,20-24). However, the particular gender-related differences between the brain regions still remain unclear. Chronic alcohol abuse leads to the damage of brain microstructure (25-29). In this study, we wanted to find out more about the effects of moderate alcohol consumption on healthy adults. Tobacco smoke toxins and nicotine are thought to be detrimental to brain tissue. Still, only little is known about their direct actions on brain microstructure. We investigated the effects of smoking on cerebral DTI parameters. Many studies have shown differences in DTI parameters between right- and left-handers (30-32). Less is know about the effects of education on cerebral white matter.

In general, the literature on possible factors affecting cerebral DTI results is relatively sparse and reported studies vary considerable in design and characteristics of the study population. Confounding factors should be routinely considered when DTI results are interpreted. Therefore better understanding of confounding factors is crucial. The aim of our study was to investigate whether age, gender, handedness, smoking, alcohol consumption and education have a confounding effect on the cerebral DTI parameters in healthy adult population. This was examined by using FA and ADC values derived from ROI analysis. Intra-observer repeatability measurements were

performed to further support the results.

Material and Methods

Subjects. The subjects were recruited among consecutive patients with ankle trauma (fracture or distension) in the Emergency Department of Tampere University Hospital. A total of 609 patients with ankle injury were screened for inclusion. The aim was to enrol five male and five female subjects to each of following age groups: (i) 18-30, (ii) 31-40, (iii) 41-50 and (iv) 51-60 years. The inclusion criteria were: (i) age 18-60 years, (ii) being resident of the university hospital district and (iii) ankle trauma. Exclusion criteria were: (i) neurological problems, (ii) psychiatric problems, (iii) history of traumatic brain injury, (iv) former neurosurgical procedure, (v) problems with hearing or vision, (vi) first language other than Finnish, (vii) contraindications to MRI and (viii) refusal to participate. Of the final sample of 40 healthy subjects, 20 (50%) were men and 20 (50%) were women. Mean ages (SD) were: whole sample 40.4 (12.3), men 39.8 (11.8) and women 41.1 (13.2) years. None of the subjects had significant structural abnormalities on conventional MRI sequences. Written informed consent was obtained from each participant. Ethics approval for the study was obtained from the Ethical Committee of the Pirkanmaa Hospital District.

Clinical Assessment. All the subjects were interviewed by phone. Interview included: (i) diagnosed medical conditions, (ii) regular daily medication, (iii) handedness, (iv) years of education, (v) smoking history (pack years), and (vi) alcohol consumption according to the Alcohol Use Disorders Identification Test (AUDIT) (33).

Magnetic Resonance Imaging. The MRI examination of the head was performed with a 3 Tesla MRI scanner (Siemens Trio, Siemens Medical Solutions, Erlangen, Germany). A 12-channel head coil and a 4-channel neck coil were used simultaneously. The MRI protocol included sagittal T1-weighted 3D IR-prepared gradient echo, axial T2-weighted turbo spin echo, conventional axial and high-resolution sagittal FLAIR (Fluid Attenuation Inversion Recovery), axial T2*-weighted, and axial SWI (Susceptibility Weighted Imaging) series. The DTI data were collected by a single-shot, spin echo-based, and diffusion-weighted echo planar imaging sequence. The parameters for

the DTI sequence were TR 5144 ms, TE 92 ms, FOV 230 mm, matrix 128 × 128, 3 averages, slice/gap 3.0/0.9 mm, voxel dimension 1.8×1.8×3.0 mm, b-factor 0 and 1000 s/mm^2, and 20 diffusion gradient orientations. A 12-channel head matrix coil was used. Evaluation of the conventional MRI scans was performed by a neuroradiologist (A.B.).

Diffusion Tensor Analysis. DTI-measurements were performed by a phycisist (U.H.) on a workstation using the commercially available software Neuro3D (Siemens Healthcare, Malvern, USA). Circular regions of interest (ROI) (19 mm²) were manually placed on color-coded axial fractional anisotropy (FA) maps and automatically transferred on the non-diffusion-weighted b_0 images and ADC maps. The ROIs were placed at the following anatomical locations: i) the cerebral peduncle (CP), ii) posterior limb of the internal capsule (PLIC) (anterior and posterior), iii) posterior part of the corona radiata (PCR) (anterior and posterior), iv) centrum semiovale (CS) (anterior, center and posterior), v) corpus callosum (CC) (genu and splenium), vi) uncinate fasciculus (UF), vii) forceps minor (FM) and viii) thalamus. The ROIs were placed taking care to avoid border areas, such as areas overlapping with cerebrospinal fluid spaces and neighboring tracts. The ROIs were placed bilaterally, with the exception of the corpus callosum, on all locations. The ROIs of the corpus callosum were drawn on the midline sagittal images. Median and mean values, as well as standard deviations, for FA and ADC were calculated. Additionally, freehand-ROI method was used with FM, UF and thalamus because of its better repeatability (2).

Statistical Analysis. The normality of the variable distributions was tested using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Constant variables (age, education years, pack years, AUDIT and AUDIT-C), FA and ADC values were analysed with the Pearson (normal distribution) and Spearman (skewed distribution) correlation coefficients. Age was grouped into four groups (18-30, 31-40, 41-50 and 51-60 years), education years into three groups (8-12, 13-15 and over 16 years), pack years into four groups (non-smokers, 0-9, 10-19 and 20-30 pack years) and both AUDIT and AUDIT-C into risk of alcohol (AUDIT, men: ≥8 and women: ≥7 points; AUDIT-C: men: ≥4 and women: ≥3 points) and non-risk users. The categorical variables were tested with the

Student's t-test (normal distribution) and the Mann-Whitney U-test (skewed distribution). The intra-observer repeatability values were assessed using the averages of intra-class correlation coefficients (ICCs) with absolute agreement. The ICC values were considered to indicate excellent agreement if they were greater than 0.8 and substantial agreement if they were from 0.60 to 0.79 (34). The statistical significance level was set to $p<0.05$ for all analyses. There was no need to control for false discovery rates as only one to two statistical tests were used to address an individual hypothesis (35). IBM SPSS Statistics 20.0 (IBM Corp. Armonk, NY, USA) was used to perform the analyses.

Results

Characteristics of the Study Sample and DTI Parameters. Detailed characteristics of the study sample are presented in Table 1. The FA and ADC results stratified by age groups are shown in Table 2 and 3.

The Relations between FA Values and Possible Confounding Factors. A significant negative correlation between FA and age was found in the right FM (r=0.39; p=0.01), left FM (r=0.41; p=0.01), right UF (r=0.33; p=0.04), left UF (r=0.41; p=0.01) and splenium of CC (r=0.32; p=0.05). In addition, the analysis showed significant differences between the age groups in the right FM (p=0.05), left UF (p=0.02) and left CP (p=0.03). The absolute FA values of the age groups are represented in table 2. FA values showed differences between the pack-year groups in the left anterior PCR (p=0.02) and both right (p=0.01) and left posterior PCR (p=0.01). In these brain regions the FA values of the heavy smokers (20-30 pack years) (n=3) were higher than the non-smokers (n=23). The same differences were not evident between the non-smokers and moderate smokers (0-9 and 10-19 pack years). The risk users of alcohol (according to AUDIT) (n=10) exhibited lower FA values than the non-risk users (n=30) in the left posterior PCR (p=0.02; d=0.06) and right thalamus (p=0.03; d=0.17), but higher values in the right FM (p=0.03; d=0.20). Lower FA values in the right thalamus (p=0.01; d=0.28) and higher FA values in the right FM (p=0.01; d=0.05) were also found in the analysis of the AUDIT-C. Apart from this, the AUDIT-C analysis showed also elevated FA values in the right posterior CS (p=0.03; d=0.37) of the risk users (n=12) compared to the non-risk users (n=28). We found significantly higher FA values in the left posterior PLIC (p=0.04) and left anterior CS (p=0.05) of the left-handers (n=1) compared to the right-handers (n=39). Significant correlations or differences between FA values and gender or education years were not found.

The Relations between ADC Values and Possible Confounding Factors. ADC values of the women (n=20) were higher than the values of the men (n=20) in the left central CS (p=0.01). In

other brain regions no gender differences were found. A positive correlation between ADC values and education was discovered in the splenium of CC (r=0.32; p=0.05). Additionally, the analysis of categorical variables showed significant differences in the splenium of CC between the education groups (p=0.01). The group with over 16 years of education (n=11) had higher ADC values (ADC=0.79) than the group of 8-12 years (ADC=0.75) (n=12) and 13-15 years (ADC=0.72) (n=17) studied. The correlation analyses revealed a significant negative correlation between ADC and pack years in the right anterior CS (r_s=0.37; p=0.02). ADC values of the heavy smokers were significantly lower than the non-smokers in three brain regions: the left anterior PCR (p=0.05), left posterior PCR (p=0.03) and right anterior CS (p=0.03). The risk users of alcohol had significantly lower ADC values than the non-risk users in the right anterior PCR (p=0.05; d=0.37) and left anterior CS (p=0.01; d=0.15). In the analysis of AUDIT-C lower ADC values among the risk users were also discovered in the left anterior CS (p=0.02; d=0.12). Significant correlations or differences between ADC values and age or handedness were not found.

Repeatability and variation of the DTI measurements. Repeatability [the intra-class correlation coefficient (ICC)] and variation [the coefficient of variation (CV) percent] results are shown in Table 4. The average ICC for FA was 0.75 and for ADC 0.78. The ICC results for FA were above 0.80 in 8 of 24 regions. For ADC, the corresponding results were 11 of 24. The best ICC for FA values was in the left UF (0.97) and for ADC values in the left thalamus (0.97). The CV percentage for FA was below 10% in 14 of 24 regions. For ADC, the CV percentage was below 10% in 23 of 24 regions. The highest variation for FA was in the right anterior CS (20.3%) and for ADC in the left anterior PCR (13.3%). For FA, the lowest variation was found in the genu of CC (2.8%) and in the splenium of CC (2.8%). The lowest variation for ADC was in the left thalamus (1.2%).

Discussion

The present study aimed to investigate the effects of possible confounding factor on DTI parameters derived from ROI-analysis in a generally healthy homogenous sample with no neurological or psychiatric diseases. Confounding factors included age, gender, handedness, smoking, alcohol consumption and education. The most consistent finding was that aging decreased FA values in the frontal brain regions. This was apparent in the right FM, left FM, right UF, left UF and splenium of CC. Also, smoking decreased ADC values in three regions. Unfortunately, the regions which were smoking-related had poor intra-observer repeatability. Regarding the other confounding factors, the results were discontinuous and no concrete conclusions could be drawn from these findings. In general, intra-observer repeatability of the DTI measurement was considered relatively good.

We found a significant negative correlation between FA and age in the right FM, left FM, right UF, left UF and splenium of CC. Respectively, the analysis also showed non-linear significant differences between the age groups in the right FM, left UF and left CP. Kochunov et al. observed that the anterior part of CC is more vulnerable to age-related decline than the posterior part (17). Many other studies have also indicated that age-related changes are most likely to happen first in the frontal areas of the brain (18,19). Hsu et al. had 145 participants, aged 30-80 years, recruited from a health screening program. Both ROI and VBA methods were used. They reported a positive correlation between FA and age in the posterior CC, CR, posterior capsula interna and superior longitudinal fasciculus (16). Yoon and colleagues used only ROI analysis and the participants of their study were 58 healthy volunteers with an age range of 22-78 years. They showed a trend of increased ADC and decreased FA values with advanced aging. Significant decrease in FA was found in numerous regions (hippocampus, temporal and frontal lobes, CS, anterior and posterior cingulum, anterior limbs of internal capsule and genu of CC) (19).

Increased FA values in men compared to women are found in many studies (16,21,23,24). Contrary to this, no gender-related differences in FA values were observed in our study. For

example, Pal et al. reported higher FA values among men in the caudate nucleus, genu and splenium of CC, PLIC and anterior limb of the internal capsula by using ROI analysis. The study population consisted of 57 women and 85 men (age: 10-52 years) (24). By using VBA-based method, Inano et al. investigated gender effects on FA, axial diffusivity and radial diffusivity. Significantly higher FA values were seen in men compared to women in many regions, for example in the splenium of CC, PLIC, CP and bilateral corona radiata. Participants in this study were 857 healthy volunteers, 310 women and 547 men, with an age range of 24.9-84.8 years (mean age 56.1 ± 9.9 years) (21). On the other hand, women had higher FA values in the CC according to Kanaan et al. (22). The groups of Pal and Inano found no significant gender differences in MD values. In our material, women had higher ADC values in the left central CS compared to men. No similar results in this particular region have been recently reported. Whereas, Lebel et al. showed higher MD values in women in the corticospinal tract, cingulum and superior longitudinal fasciculus (23).

FA values of the heavy smokers were higher than those of the non-smokers in the left anterior PCR and both right and left posterior PCR. The heavy smokers had also lower ADC values compared to non-smokers in the left anterior PCR, left posterior PCR and right anterior CS. Additionally, a significant negative correlation between ADC and pack years was found only in the right anterior CS. Although significant group differences were found in relation to smoking, the association to DTI parameters was non-linear. Therefore, no clear dose-dependence between DTI markers and smoking could be concluded. It is well documented that gray matter volume and density in smokers are smaller than in non-smokers. Three DTI studies have demonstrated reduced gray matter density in the lateral prefrontal cerebral cortex (36-38). The findings of gray matter volume or density in other brain regions are mixed. Yu et al. found increased white matter density in the putamen and cingulate cortex among smokers by using VBA. Their results were based on the data of 16 cigarette smokers and 16 matched healthy non-smoking controls (39). Moreover, Paul and co-authors reported higher FA values in smokers (n=10) compared to non-smokers (n=10) in the CC by using ROI analysis (40).

We found that the risk users of alcohol had lower FA values than the non-risk users in the left posterior PCR and right thalamus, but higher values in the right FM and right posterior CS. Lower ADC values among the risk users compared to the non-risk users were found in the right anterior PCR and left anterior CS. Chronic alcohol abuse leads to reduced white and gray matter volume (25-29). However, little is known about the effects of moderate alcohol consumption. McQueeny et al. studied white matter integrity among adolescent binge drinkers (n=14) compared to controls (n=14) without a history of a binge drinking episodes. They found lower FA values in 18 white matter areas, including the CC, CR and limbic projection fibres, relative to controls by using tract-based spatial statistics (41). Pfefferbaum et al. concluded that chronic alcohol consumption leads to the demyelination of white matter tracts (29). Their earlier studies also showed that the CC is very vulnerable to alcohol (27,28).

To our knowledge, only few studies have examined cerebral DTI metrics in relation to education. Piras et al. reported a negative correlation between MD values and education in the bilateral hippocampi, right caudate, bilateral thalami and bilateral putamen, but it is unclear if the relationship was mediated by age. The study population was consisted of 150 healthy subjects (age between 18 and 65 years) (42). Teipel and co-authors reported that higher white matter integrity was associated with more years of education among 18 healthy controls (mean age 66.2 years) in the medial temporal lobe areas, fusiform gyrus, insula, superior temporal gyrus and lingual gyrus (43). In our study, the most educated subjects (16 years or more) had the highest ADC values in the splenium of CC compared to the other groups. Moreover, a positive correlation between ADC and education was discovered in the same brain region. None of the previous studies have observed similar results. Generally, higher ADC is indicative of neuron loss. Therefore, our result seems contradictional and is left without a logical explanation.

Left cerebral hemisphere plays a dominant role among right-handers and it has been well demonstrated, for instance, by comparing the sizes of the hemispheres (32) and FA values of white matter between precentral gyri (30). According to Powell and his colleagues, right-handers have

greater leftward FA asymmetry than left-handers. However, the left-handers showed also leftward asymmetry in many white matter regions (31). By using ROI analysis, Westerhausen et al. discovered that left-handedness was related to higher anisotropy and lower diffusion in the CC (32). This can be related to an idea that left-handers have a more symmetrical brain than right-handers. We found significantly higher FA values in the left posterior PLIC and left anterior CS of the left-handers compared to the right-handers. The group of left-handers consisted of only one subject, so no concrete conclusion can be drawn from these findings.

Intra-class correlation analysis revealed that the best repeatability for both FA and ADC values was in the UF, FM, thalamus and CC. ADC values had better overall repeatability and lower variation than FA values. The low ICC values were found for FA in the CS and the variation was highest for FA in the PCR and CS.

The main limitation of our study was the low statistical power in some subgroup analysis due to the small sample sizes. In addition, strict exclusion criteria limit the generalizability of the results. Also, our sample only included patients with moderate alcohol consumption (AUDIT≤10). If the AUDIT score exceeded 10 points, the patient's alcohol consumption habits were considered generally detrimental and the patient was excluded from the study. Thus, the study design did not entitle us to examine the effects of heavy alcohol usage.

Despite the aforementioned limitations, the current study has several strengths. First, we minimized the effects neurological and psychiatric diseases on DTI parameters by applying thorough exclusion criteria and patient screening. Second, the performed data collection was inclusive and detailed. Third, the DTI measurement protocol included the majority of the brain regions studied in DTI literature.

In conclusion, age and smoking should be noted as considerable confounding factor in ROI-based DTI-analysis. More research on the effects of gender, handedness, alcohol consumption and education is needed. Replication of this study design using other DTI methods (e.g., tract-based spatial statictics, tractography) is encouraged.

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Author Disclosure Statement

The authors alone are responsible for the content and writing of the paper.

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Table legends

Table 1. Detailed characteristics of the study sample.

Table 2. FA values by region of interest and stratified by age.

Table 3. ADC values by region of interest and stratified by age.

Table 4. Intra-class correlations of FA and ADC measurements.

Table 1. Detailed characteristics of the study sample

	Mean	**SD**	**Median**	**Range**
Age (years)	40.43	12.34	41.65	38.50
Education years	14.14	2.84	14.00	13.00
Pack years	5.08	8.54	0.00	30.00
AUDIT (0-40p)	4.80	2.92	5.00	11.00
AUDIT-C (0-12p)	4.20	2.32	4.00	9.00

Table 2. FA values by region of interest and stratified by age. $0 \leq FA \leq 1$.

		Whole sample		18-30 years		31-40 years		41-50 years		51-60 years	
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
CP	right	0.80	0.54	0.82	0.04	0.77	0.06	0.81	0.06	0.80	0.05
	left	0.80	0.06	0.84	0.04	0.77	0.05	0.81	0.04	0.78	0.07
Anterior PLIC	right	0.70	0.43	0.72	0.03	0.70	0.04	0.69	0.04	0.70	0.06
	left	0.70	0.45	0.71	0.04	0.72	0.05	0.69	0.04	0.70	0.05
Posterior PLIC	right	0.70	0.04	0.73	0.03	0.69	0.03	0.70	0.03	0.69	0.05
	left	0.71	0.05	0.73	0.06	0.71	0.04	0.71	0.03	0.68	0.04
Anterior PCR	right	0.45	0.07	0.46	0.05	0.42	0.08	0.46	0.06	0.45	0.07
	left	0.51	0.09	0.52	0.09	0.46	0.09	0.57	0.10	0.49	0.08
Posterior PCR	right	0.52	0.07	0.54	0.07	0.49	0.07	0.53	0.06	0.52	0.08
	left	0.55	0.07	0.55	0.06	0.54	0.05	0.55	0.08	0.55	0.07
Anterior CS	right	0.63	0.08	0.62	0.09	0.65	0.07	0.60	0.07	0.63	0.08
	left	0.61	0.08	0.63	0.09	0.60	0.08	0.61	0.07	0.59	0.09
Central CS	right	0.59	0.10	0.65	0.07	0.57	0.10	0.61	0.07	0.53	0.12
	left	0.61	0.09	0.61	0.07	0.65	0.10	0.57	0.10	0.61	0.08
Posterior CS	right	0.57	0.06	0.56	0.07	0.58	0.06	0.54	0.07	0.59	0.04
	left	0.56	0.06	0.58	0.06	0.55	0.05	0.56	0.07	0.57	0.06
UF	right	0.56	0.06	0.60	0.06	0.55	0.05	0.55	0.07	0.53	0.05
	left	0.56	0.06	0.60	0.07	0.56	0.05	0.53	0.05	0.53	0.06
FM	right	0.51	0.07	0.55	0.07	0.51	0.06	0.47	0.06	0.50	0.07
	left	0.48	0.05	0.52	0.05	0.49	0.03	0.47	0.06	0.46	0.06
Thalamus	right	0.32	0.03	0.32	0.03	0.31	0.03	0.33	0.02	0.32	0.02
	left	0.32	0.03	0.32	0.05	0.31	0.03	0.33	0.02	0.31	0.02
Genu of CC		0.82	0.05	0.85	0.03	0.81	0.06	0.80	0.05	0.82	0.04
Splenium of CC		0.86	0.04	0.88	0.04	0.87	0.03	0.85	0.04	0.85	0.05

Table 3. ADC values by region of interest and stratified by age. ADCx$10^{-3}$$mm^2$/s.

		Whole sample		18-30 years		31-40 years		41-50 years		51-60 years	
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
CP	right	0.77	0.07	0.79	0.07	0.77	0.07	0.74	0.08	0.77	0.06
	left	0.74	0.06	0.75	0.03	0.76	0.05	0.71	0.05	0.73	0.09
Anterior PLIC	right	0.72	0.04	0.71	0.03	0.72	0.04	0.71	0.03	0.72	0.06
	left	0.70	0.04	0.71	0.04	0.70	0.04	0.70	0.04	0.69	0.03
Posterior PLIC	right	0.72	0.03	0.72	0.03	0.72	0.01	0.71	0.03	0.73	0.03
	left	0.70	0.03	0.72	0.03	0.71	0.02	0.70	0.02	0.70	0.04
Anterior PCR	right	0.69	0.05	0.68	0.04	0.70	0.02	0.68	0.06	0.71	0.05
	left	0.65	0.08	0.63	0.08	0.68	0.04	0.64	0.12	0.65	0.08
Posterior PCR	right	0.73	0.05	0.70	0.06	0.74	0.04	0.75	0.05	0.74	0.04
	left	0.71	0.05	0.71	0.03	0.73	0.04	0.70	0.05	0.70	0.06
Anterior CS	right	0.72	0.04	0.71	0.05	0.73	0.03	0.72	0.06	0.71	0.03
	left	0.72	0.05	0.72	0.05	0.72	0.03	0.70	0.05	0.72	0.05
Central CS	right	0.73	0.04	0.73	0.04	0.72	0.04	0.73	0.05	0.72	0.03
	left	0.71	0.05	0.69	0.05	0.71	0.03	0.71	0.07	0.72	0.03
Posterior CS	right	0.73	0.04	0.73	0.03	0.73	0.03	0.74	0.04	0.74	0.06
	left	0.72	0.05	0.69	0.04	0.74	0.04	0.71	0.06	0.73	0.05
UF	right	0.79	0.04	0.77	0.04	0.80	0.04	0.79	0.04	0.79	0.05
	left	0.79	0.04	0.77	0.03	0.80	0.03	0.79	0.04	0.80	0.05
FM	right	0.76	0.03	0.74	0.04	0.77	0.03	0.77	0.03	0.77	0.03
	left	0.77	0.04	0.76	0.04	0.77	0.03	0.78	0.03	0.79	0.04
Thalamus	right	0.77	0.04	0.79	0.07	0.76	0.03	0.76	0.04	0.77	0.03
	left	0.76	0.03	0.76	0.02	0.75	0.03	0.75	0.03	0.76	0.03
Genu of CC		0.77	0.05	0.76	0.05	0.78	0.04	0.78	0.05	0.76	0.03
Splenium of CC		0.75	0.06	0.75	0.06	0.77	0.08	0.74	0.06	0.74	0.04

Table 4. Intra-class correlation and variation coefficients of FA and ADC measurements. ADCx$10^{-3}$$mm^2$/s; $0 \leq FA \leq 1$.

		FA			ADC		
		ICC	p	CV (%)	ICC	p	CV (%)
CP	right	0.73	< 0.001	6.2	0.63	0.002	9.0
	left	0.67	< 0.001	6.9	0.75	< 0.001	6.7
Anterior PLIC	right	0.88	< 0.001	3.8	0.81	< 0.001	4.2
	left	0.77	< 0.001	5.9	0.78	< 0.001	4.2
Posterior PLIC	right	0.73	< 0.001	4.8	0.83	< 0.001	3.0
	left	0.75	< 0.001	5.9	0.65	0.001	3.9
Anterior PCR	right	0.69	< 0.001	14.6	0.85	< 0.001	5.6
	left	0.76	< 0.001	15.5	0.58	0.004	13.3
Posterior PCR	right	0.64	0.001	15.9	0.77	< 0.001	5.3
	left	0.79	< 0.001	11.9	0.62	0.001	6.4
Anterior CS	right	0.40	0.028	20.3	0.82	< 0.001	4.7
	left	0.68	< 0.001	14.2	0.61	0.003	6.7
Central CS	right	0.59	< 0.001	18.0	0.70	< 0.001	5.9
	left	0.58	0.001	17.4	0.64	< 0.001	7.8
Posterior CS	right	0.62	0.001	14.1	0.73	< 0.001	4.9
	left	0.45	0.019	13.1	0.71	< 0.001	6.0
UF	right	0.94	< 0.001	5.1	0.91	< 0.001	3.3
	left	0.97	< 0.001	4.5	0.93	< 0.001	2.4
FM	right	0.88	< 0.001	8.0	0.81	< 0.001	3.5
	left	0.88	< 0.001	8.3	0.92	< 0.001	2.9
Thalamus	right	0.76	< 0.001	7.0	0.79	< 0.001	4.1
	left	0.88	< 0.001	6.5	0.97	< 0.001	1.2
Genu of CC		0.94	< 0.001	2.8	0.94	< 0.001	2.8
Splenium of CC		0.92	< 0.001	2.8	0.93	< 0.001	4.0