

## Cingulum fiber diffusivity and CSF T-tau in patients with subjective and mild cognitive impairment

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### Abstract

Diffusion tensor imaging (DTI) and CSF biomarkers are useful diagnostic tools to differentiate patients with mild cognitive impairment (MCI) from normal controls, and may help predict conversion to dementia. Total Tau protein (T-tau) and DTI parameters are both markers for axonal damage, thus it is of interest to determine if DTI parameters are associated with elevated CSF T-tau levels in patients with cognitive impairment. For this purpose, patients with subjective cognitive impairment (SCI) and MCI were recruited from a university based memory clinic.

Regions of interest were used to determine fractional anisotropy (FA), radial diffusivity (DR) and axial diffusivity (DA) in known white matter tracts in patients with MCI ( $n = 39$ ) and SCI ( $n = 8$ ) and 26 cognitively healthy controls. Significant lower FA and higher DR values were observed in patients with pathological vs. patients with normal CSF T-tau levels and vs. controls in left posterior cingulum fibers. T-tau values were negatively correlated with FA and positively correlated with DR values in the posterior cingulum fibers.

Cingulum fiber diffusivity was related to T-tau pathology in SCI/MCI patients and altered DR may suggest that loss of myelin contributes to early white matter changes in patients at risk of developing Alzheimer's disease (AD).

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### 1. Introduction

Individuals with mild cognitive impairment (MCI) (Petersen et al., 1999, 2001) are at risk of developing Alzheimer's disease (AD) and approximately 50% will con-

vert to AD within 5 years (Petersen, 2004). MCI etiology is heterogeneous and in order to understand mechanisms for disease development and enable development of disease modifying drugs it is important to recognize the underlying pathology at an early stage (DeKosky and Marek, 2003). Recent studies suggest that subjective cognitive impairment (SCI) may be a pre-MCI stage and the first detectable stage in the SCI–MCI–AD development (for review see Reisberg and Gauthier, 2008). If this disease process is a continuum, both CSF biomarkers and advanced neuroimaging should

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be applied also in the study of SCI patients to detect early underlying pathology.

CSF biomarkers (tau and beta-amyloid proteins) predict conversion from MCI to AD with high sensitivity and specificity (Diniz et al., 2007; Hansson et al., 2006), and may contribute to early detection of AD (de Leon et al., 2007). The Tau protein is a microtubule-associated protein mainly located in neuronal axons and is important for microtubuli stabilization and axonal maintenance (Drubin et al., 1988). Elevated CSF levels of total Tau protein (T-tau) have been observed in different neurodegenerative diseases and are probably markers for axonal damage (Sunderland et al., 2003; Sussmuth et al., 2001; Teunissen et al., 2005).

Diffusion tensor imaging (DTI) may be used to quantify white matter integrity (Le Bihan et al., 2001) and may reveal white matter changes not detectable with conventional MRI (Deo et al., 2006; Taylor et al., 2007). Fractional anisotropy (FA), calculated from the diffusion tensor eigenvalues, is a measure of directional diffusivity (Basser et al., 1994; Le Bihan et al., 2001) and reduced FA has been associated with age-related cognitive decline (Charlton et al., 2006). Reduced FA has also been observed in posterior cingulum fibers in MCI patients compared to normal controls (Fellgiebel et al., 2005; Zhang et al., 2007). The diffusion tensor eigenvalues may be separated into components which describe diffusivity parallel ( $\lambda_1$ ) or perpendicular ( $\lambda_2$  and  $\lambda_3$ ) to the axonal tracts (Basser et al., 1994; Xue et al., 1999). Axial diffusivity (DA, parallel to axon tracts) and radial diffusivity (DR, perpendicular to axon tracts) may be helpful to better describe the underlying pathology of white matter alterations reflected by FA. After experimental ischemia, reduced DA is followed by increased DR mirroring the sequence of Wallerian degradation of axons and myelin components in the CNS (George and Griffin, 1994a,b; Sun et al., 2008). It has been suggested that decreased DA may be related to axonal loss and increased DR may be associated with loss of myelin (Pierpaoli et al., 2001; Song et al., 2002, 2003), though parts of the DA and FA responses may be transient (Concha et al., 2006). Experimental evidence from contusion injuries also suggests that DR changes are sensitive to secondary changes (beyond the site of the primary histologically defined lesion) (Budde et al., 2007).

Few earlier studies have examined directional diffusivity (DR and DA) in patients with cognitive impairment, and so far the findings have been inconclusive. Huang et al. (2007) reported findings consistent with axonal degeneration (altered DA) in the temporal lobe of AD and MCI patients, whereas Choi et al. (2005) focused on frontal regions and found signs of altered myelination (DR) in frontal white matter of early AD patients. To our knowledge, no prior study has investigated associations between directional white matter diffusivity and CSF biomarkers in MCI patients. In this study selected regions of interest (ROI) were chosen in areas associated with the AD disease process. Corpus callosum (CC) was chosen as neocortical connections may be involved in the AD disease process (Braak et al., 1999) and posterior cingu-

lum fibers and forceps major ROI were chosen as changes in these posterior areas have been associated with MCI and early AD. As a part of the Papez-circuit (Papez, 1995), the posterior cingulum fibers play an important role in memory function as they connect the posterior cingulate gyrus with the medial temporal lobe (Buckner et al., 2005; Catani et al., 2002; Crosby et al., 1962). Studies using structural and functional imaging techniques have shown alterations of the posterior cortex including posterior cingulate gyrus as well as of the medial temporal lobe in patients with MCI and AD (Convit et al., 1997; Du et al., 2001; Jack et al., 1999).

This study aimed (1) to compare directional white matter diffusivity between SCI/MCI patients with and without CSF T-tau pathology and cognitively healthy controls, and (2) to study associations between directional white matter diffusivity and CSF T-tau levels in SCI/MCI patients. As Tau pathology may reflect axonal damage, we hypothesized that white matter diffusivity changes observed in SCI/MCI patients would be more visible in patients with elevated CSF T-tau levels than in patients with normal T-tau levels.

## 2. Methods

### 2.1. Subjects

Forty-seven patients (mean age 61.4 [43–77] years, SD 7.9; 21 females; mean Mini-Mental State Exam (MMSE) (Folstein et al., 1975) 27.8 [23–30], SD 1.7) were recruited from a university-based memory clinic. Inclusion criteria were subjective memory impairment, preserved general intellectual function, no or very mild ADL problems, symptoms lasting  $\geq 6$  months, and Clinical Dementia Rating (CDR)=0.5 (Morris, 1993). Criteria for exclusion were established psychiatric disorder, cancer, drug abuse, solvent exposure or anoxic brain damage. All patients were diagnosed with either SCI or MCI (Gauthier et al., 2006; Petersen et al., 1999; Reisberg and Gauthier, 2008) and had Global Deterioration Scale 2 (8 patients) or 3 (39 patients) (Auer and Reisberg, 1997; Reisberg et al., 1988) as determined from a clinical interview and screening tests. Screening tests included parameters 13–20 (memory, disorientation, abstract thinking, visuospatial ability, language, sensory aphasia, visual agnosia, and apraxia) from the stepwise comparative status analysis (STEP, Wallin et al., 1996; Edman et al., 2001), word fluency, interference, and numeral-letter items from the I-flex (Royall et al., 1992), and items from the Neurobehavioral Cognitive Status Examination (Cognistat, Kiernan et al., 1987), as well as MMSE. Patients with results above cutoff on these screening tests were diagnosed with SCI (GDS 2), whereas patients scoring below cutoff were classified as MCI (GDS 3) (Reisberg and Gauthier, 2008). One patient with GDS = 3 had MMSE = 23, but was included as she had normal employment and was self-sufficient. According to current criteria for MCI, objectively verifiable memory impairment or deficits in other cognitive domains must be

present (Petersen et al., 1999; Winblad et al., 2004). Patients with early cognitive deficits may fall into either GDS groups 2 and 3, e.g. based on pre-morbid cognitive capacity and the fact that cutoffs for cognitive tests are group-based and not individualized. In order not to exclude patients with very early disease, CDR 0.5 patients with screening test results above cutoff, corresponding to criteria for SCI (or GDS 2), were also included. “CDR 0.5/uncertain dementia” (Morris et al., 2001) and “pre-MCI” (Storandt et al., 2006) are previously used nomenclature for the latter patient group.

Provided a GDS score of 1 (a history of and clinically established normality with regard to memory, emotionality and tempo), spouses of participating patients were included as controls ( $N=26$ ). Controls had GDS 1 as determined by a clinical interview, but were not tested formally.

The study was approved by the South-Eastern Norway ethical committee for medical research, and informed consent was obtained from all subjects included in the study.

Age dependent clinical cut-off values for CSF T-tau (Sjogren et al., 2001) were used to define the SCI/MCI patients into two groups: SCI/MCI<sub>aTau</sub> if abnormal T-tau values and SCI/MCI<sub>nTau</sub> if normal T-tau values (see below).

Due to practical reasons two MRI scanners were used (see below). The mean time between lumbar puncture and MRI scan was 3.0 months (SD=3.4). Table 1 shows the characteristics of patients and controls.

## 2.2. MRI acquisition

The following structural images were acquired: axial fluid-attenuated inversion recovery (FLAIR), T2-weighted fast spin echo (FSE) and T1-weighted 3D magnetization prepared, gradient echo (MPRAGE) (specified elsewhere, Fjell et al., 2008). Diffusion tensor images were acquired using a 2D spin echo planar imaging (EPI) sequence diffusion weighting applied along 12 noncollinear directions with two (site I) and five (site II) repetitions per direction to increase the signal-to-noise ratio. In addition, two (site I) and five (site II) images without diffusion weighting ( $b=0$ ) were acquired. Two different Siemens 1.5T MRI scanners were used. Site I—Siemens Symphony: acquisition matrix =  $128 \times 128$ , pixel size =  $1.8 \text{ mm} \times 1.8 \text{ mm}$ , TE = 131 ms, TR = 4300 ms,  $b$  value =  $700 \text{ s/mm}^2$ , section spacing/thickness =  $1.5 \text{ mm}/5 \text{ mm}$ . Site II—Siemens Espree: acquisition matrix =  $192 \times 192$ , pixel size =  $1.2 \text{ mm} \times 1.2 \text{ mm}$ , TE = 117 ms, TR = 6100 ms,  $b$  value =  $750 \text{ s/mm}^2$ , section spacing/thickness =  $0.9 \text{ mm}/3 \text{ mm}$ .

## 2.3. DTI data processing and ROI analysis

The DTI data were co-registered to the MPRAGE images using Statistical Parametric Mapping (SPM5, Wellcome Trust Centre for Neuroimaging, London, UK). DTI-MPRAGE co-registration was performed using the  $b=0$  scans as the source images and the resulting transformation matrix was then applied to the calculated parametric

DTI maps. All DTI analysis was performed using the nordicICE Basis and Diffusion Modules (NordicImagingLab AS, Bergen, Norway). In addition to ADC, FA and individual eigenvalue images, color-coded eigenvector (cDTI) maps were generated where pixel color and intensity reflect the principal diffusion direction and FA magnitude, respectively.

Typical eddy current artefacts (areas of artificially high FA values) were not observed in any of the objects and we found that the built-in eddy current compensation methods on the scanners effectively eliminated any significant eddy current artefacts in the images. Applying off-line eddy current correction using the method implemented in nordicICE (Rohde et al., 2005) did not lead to a further reduction in eddy current artefacts. The raw data were further inspected for significant motion, but as this was not observed in any of the included objects motion correction was not applied.

Regions of interest (ROI) values were extracted from all processed images (FA, ADC,  $\lambda_1$ ,  $\lambda_2$ ,  $\lambda_3$ ). DR was calculated as  $(\lambda_2 + \lambda_3)/2$  for each ROI.

White matter segmentations were created from the co-registered MPRAGE sequence using the segmentation module in SPM5. The white matter masks as well as the cDTI maps were used for orientation to avoid partial CSF or grey matter volume and ROI were placed in the part of the fiber tract with the highest signal intensity. To ensure the right anatomical placement of the ROI between subjects, images were compared. Six elliptical ROI were manually drawn in one slice in the FA map in the following known white matter tracts: left and right in the posterior cingulum fibers (site I/II:  $25.8/27.3 \text{ mm}^2$ ), genu and splenium of the corpus callosum (site I/II:  $32.3/34.4 \text{ mm}^2$ ), and the left and right major forceps (site I/II:  $25.8/27.3 \text{ mm}^2$ ). Fig. 1 illustrates the placement of the ROI in the color-coded map and Table 2 shows the mean ROI values for the whole sample. ROI were placed by one rater (VS). To determine the intrarater reliability, 150 ROI ( $10 \text{ ROI} \times 15 \text{ patients}$ ) were placed twice and the intrarater correlation coefficient (Intraclass correlation coefficient) was calculated to 0.89.

White matter hyperintensities (WMH) were quantified with a semi-automated method in the nordicICE Basis Module. In the FLAIR images, pixel values in white matter higher than 2 SD above mean pixel value of the respective slice were defined as WMH. The total WMH area in all slices were added together and multiplied with slice thickness to obtain total WMH volume (mL).

## 2.4. Lumbar puncture and CSF analysis

All patients had lumbar puncture (LP) and CSF T-tau was routinely examined with a commercially available kit (Innogenetics, Belgium) adapted to a Tecan Robotic Microplate 150 Processor (Tecan AG, Switzerland). Clinical cut-off values for CSF T-tau were used to define the patients into SCI/MCI<sub>aTau</sub> ( $N=12$ ) and SCI/MCI<sub>nTau</sub> ( $N=35$ ). CSF T-tau level was considered abnormal if: T-tau  $\geq 300 \text{ ng/L}$  for patients under 50 years,  $\geq 450 \text{ ng/L}$  for patients from 50 to

Table 1  
Characteristics of patients and controls.

	SCI/MCI <sub>a</sub> Tau		SCI/MCI <sub>n</sub> Tau		Controls	
	S1 (n=5)	S2 (n=7)	S1 (n=16)	S2 (n=19)	S1 (n=13)	S2 (n=13)
Age	66.8 (5.5)	64.1 (10.4)	59.8 (8.6)	60.3 (6.4)	62.6 (7.4)	62.2 (8.5)
Sex: male/female (n)	3/2	3/4	11/5	9/10	5/8	8/5
Symptom duration, years	1.7 (1.0)	1.9 (0.7)	2.5 (2.5)	2.4 (1.7)	NA	NA
WML load (mL)	4.3 (3.4)	1.1 (1.0)	2.9 (6.3)	3.9 (6.2)	0.9 (1.4)	2.7 (3.0)
MMSE	27.2 (1.6)	27.3 (2.1)	27.8 (2.0)	28.3 (1.3)	NA	NA
GDS	3.0 (0.0)	2.9 (0.4)	2.9 (0.3)	2.7 (0.5)	1.0 (1.0)	1.0 (1.0)

The table shows the characteristics of all patients and controls, also with respect to MRI site. Numbers are mean (SD) if not otherwise specified. A significant difference in WMH volume between sites I and II in the SCI/MCI<sub>a</sub>Tau group (mean difference = 3199 mL, 95% CI of the difference = 207; 6190,  $p=0.038$ ) was observed. The 12 patients with pathological CSF T-tau values had a borderline significant higher mean age than SCI/MCI<sub>n</sub>Tau ( $df=45$ ,  $p=0.055$ ) and a non-significant higher mean age compared to controls ( $df=36$ ,  $p=0.33$ ). No further significant group differences were observed.

SCI/MCI<sub>a</sub>Tau = SCI/MCI patients with pathological CSF T-tau levels.

SCI/MCI<sub>n</sub>Tau = SCI/MCI patients with normal CSF T-tau levels.

S1 = MRI site I, S2 = MRI site II.

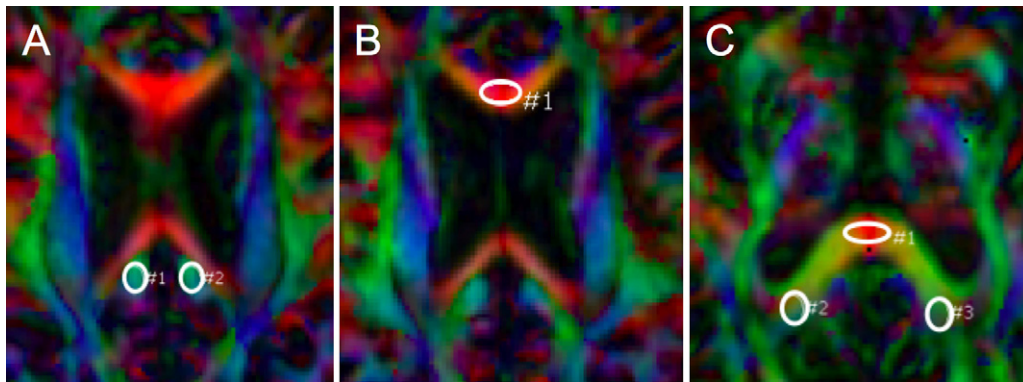


Fig. 1. Illustration of region of interest (ROI) placements in the color-coded (cDTI) map. Cingulum fibers (A), genu corpus callosum (B), splenium corpus callosum and forceps major (C).

Table 2  
Directional diffusivity ROI values for SCI/MCI patients with and without CSF T-tau pathology and controls.

	SCI/MCI <sub>a</sub> Tau (n=12)	SCI/MCI <sub>n</sub> Tau (n=35)	Controls (n=26)
Cingulum right FA	0.56 (0.10)	0.61 (0.07)	0.60 (0.09)
Cingulum left FA	0.56 (0.09) <sup>#, §</sup>	0.61 (0.08)	0.62 (0.10)
Genu CC FA	0.67 (0.09) <sup>*</sup>	0.70 (0.09)	0.72 (0.07)
Splenium CC FA	0.76 (0.07)	0.75 (0.08)	0.78 (0.05)
Forceps major right FA	0.54 (0.11)	0.55 (0.08)	0.58 (0.06)
Forceps major left FA	0.52 (0.08) <sup>*</sup>	0.55 (0.07)	0.60 (0.09)
Cingulum right DR	0.50 (0.11)	0.45 (0.09)	0.46 (0.09)
Cingulum left DR	0.54 (0.10) <sup>#, §</sup>	0.47 (0.09)	0.46 (0.12)
Genu CC DR	0.54 (0.23) <sup>*</sup>	0.49 (0.19)	0.44 (0.13)
Splenium CC DR	0.40 (0.17)	0.39 (0.14)	0.36 (0.09)
Forceps major right DR	0.57 (0.12)	0.57 (0.10)	0.54 (0.07)
Forceps major left DR	0.59 (0.10) <sup>*</sup>	0.56 (0.08)	0.53 (0.09)
Cingulum right DA	1.30 (0.13)	1.35 (0.12)	1.34 (0.14)
Cingulum left DA	1.41 (0.11)	1.40 (0.13)	1.43 (0.12)
Genu CC DA	1.85 (0.32)	1.84 (0.22)	1.81 (0.19)
Splenium CC DA	1.90 (0.33)	1.84 (0.19)	1.89 (0.22)
Forceps major right DA	1.45 (0.20)	1.45 (0.16)	1.48 (0.14)
Forceps major left DA	1.44 (0.15)	1.45 (0.14)	1.54 (0.21)

Numbers are mean (SD). Units for DR and DA is  $10^{-3}$  mm<sup>2</sup>/s.

FA = fractional anisotropy, DR = radial diffusivity, DA = axial diffusivity, and CC = corpus callosum.

<sup>\*</sup> Statistically significant vs. controls ( $p < 0.05$ ).

<sup>#</sup> Statistically significant vs. controls ( $p < 0.01$ ).

<sup>§</sup> Statistically significant vs. SCI/MCI<sub>n</sub>Tau ( $p < 0.05$ ) after correcting for age, sex, WMH volume, and MRI scanner (Mann–Whitney  $U$ -test, see Fig. 2).

69 years, and  $\geq 500$  ng/L for patients from 70 years and above (Sjogren et al., 2001).

2.5. Statistical analysis

The Statistical Package for Social Sciences (SPSS for Mac, Version 16.0, SPSS, Chicago, IL) was used for all statistical analyses.

Student's *t*-tests and Chi-squared test were used to test differences in group demographics and the Mann–Whitney *U*-test was used to compare differences in directional diffusivity between the two patient groups (SCI/MCI<sub>aTau</sub> and SCI/MCI<sub>nTau</sub>) and controls. As two different MRI scanners were used, ROI values from the posterior cingulum fibers were first compared between the diagnostic groups for each MRI site separately. Age was regressed out and the standardized residuals (*z*-scores) were compared for each scanner separately.

Next, the whole sample was analysed using the same method. Age, sex, WMH volume, and MRI scanner were regressed out and the standardized residuals for all ROI values were used in the analyses. WMH volume was included in the analyses as WMH may cause loss of directional diffusivity (Jones et al., 1999).

To analyse the relationship between CSF T-tau values and diffusion parameters for white matter damage, FA, DR, and DA ROI values were used as dependent variables in backward linear regression analysis as a function of CSF T-tau values. Age, sex, WMH volume, and MRI scanner were included in the model as possible confounding factors. All *p*-values were finally Bonferroni corrected (6 $\times$ ) as multiple ROI analyses were performed.

3. Results

Table 1 shows the characteristics of the included patients and controls from both MRI sites. We found no significant demographic differences between groups scanned at sites I and II, or the three diagnostic groups (data not shown). A significant difference in WMH volume between sites I and II in the SCI/MCI<sub>aTau</sub> group (Student's *t*-test: mean difference = 3199 mL, 95% CI of the difference = 207; 6190, *p* = 0.038) was observed. The 12 patients with pathological CSF T-tau values had a borderline significant higher mean age (mean age [SD] = 65.2 [8.4]) than SCI/MCI<sub>nTau</sub> (mean age [SD] = 60.1 [7.3], *df* = 45, *p* = 0.055) and a non-significant higher mean age compared to controls (mean [SD] = 62.4 [7.8], *df* = 36, *p* = 0.33). The SCI/MCI<sub>aTau</sub> and SCI/MCI<sub>nTau</sub> patients did not differ in MMSE performance or symptom duration.

The SCI/MCI<sub>aTau</sub> patients had significantly lower FA and higher DR values in the posterior cingulum fibers compared to controls (see below). These findings were consistent across the samples from the two different MRI sites (Supplementary Fig. 1). Table 2 shows the diffusion parameters FA, DR, and

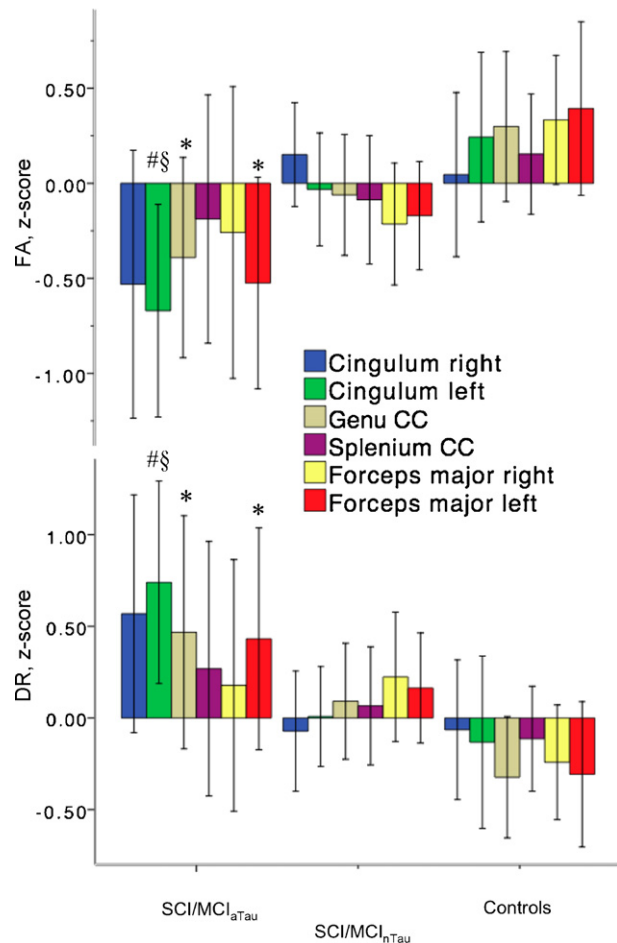


Fig. 2. Standardized residuals (*z*-scores) after correcting for age, sex, WMH volume, and MRI scanner. SCI/MCI<sub>aTau</sub> had significantly higher DR in genu corpus callosum, left forceps major, and left posterior cingulum compared to controls and higher DR in the left posterior cingulum compared to SCI/MCI<sub>nTau</sub> (Mann–Whitney *U*-test). \*Statistically significant vs. controls (*p* < 0.05, uncorrected). #Statistically significant vs. controls (*p* < 0.01, uncorrected). \$Statistically significant vs. SCI/MCI<sub>nTau</sub> (*p* < 0.05, uncorrected). FA = fractional anisotropy; DR = radial diffusivity.

DA in the ROIs and Fig. 2 shows the corrected standardized residuals corrected for age, sex, WMH volume, and MRI site for the three diagnostic groups.

Group analyses with the Mann–Whitney *U*-test showed that SCI/MCI patients with CSF T-tau pathology had significantly lower FA in genu corpus callosum (Mann–Whitney *U* score = 91, *z*-score = -2.04, *p* = 0.042), left forceps major (Mann–Whitney *U* score = 84, *z*-score = -2.26, *p* = 0.023), and left posterior cingulum fibers (Mann–Whitney *U* score = 66, *z*-score = -2.83, *p* = 0.004) compared to controls. SCI/MCI<sub>aTau</sub> had significantly lower FA in the left posterior cingulum (Mann–Whitney *U* score = 129, *z*-score = -1.98, *p* = 0.048) also when compared to SCI/MCI<sub>nTau</sub>.

As can be seen in Table 2, no differences in axial diffusion between the groups were observed.

SCI/MCI<sub>aTau</sub> had significantly higher DR in genu corpus callosum (Mann–Whitney *U* score = 91, *z*-score = -2.04,

Table 3  
The relationship between CSF T-tau values and directional diffusivity ROI values.

	FA	<i>p</i>	DR	<i>p</i>	DA	<i>p</i>
Cingulum right	−0.38	0.016	0.40	0.007*	−0.095	0.60
Cingulum left	−0.35	0.029	0.40	0.005*	−0.001	0.99
Genu CC	−0.022	0.87	0.057	0.62	0.11	0.30
Splenium CC	−0.016	0.92	0.057	0.69	0.11	0.42
Forceps major right	−0.021	0.89	0.020	0.89	−0.026	0.89
Forceps major left	−0.15	0.34	0.14	0.41	−0.035	0.85

Numbers show the standardized regression coefficients ( $\beta$ ). FA, DR, and DA ROI values were used as dependent variables in backward linear regression analysis as a function of CSF T-tau values. Age, sex, WMH volume, and MRI scanner were regressed out. *p*-Values are uncorrected.

\*  $p < 0.05$ , after Bonferroni correction ( $6 \times$ ).

$p = 0.042$ ), left forceps major (Mann–Whitney  $U$  score = 92,  $z$ -score =  $-2.01$ ,  $p = 0.044$ ), and left posterior cingulum (Mann–Whitney  $U$  score = 72,  $z$ -score =  $-2.64$ ,  $p = 0.007$ ) compared to controls and higher DR in the left posterior cingulum (Mann–Whitney  $U$  score = 106,  $z$ -score =  $-2.54$ ,  $p = 0.011$ ) compared to SCI/MCI<sub>nTau</sub>, indicating that the observed altered FA in MCI<sub>aTau</sub> was due to changes in radial diffusion.

After Bonferroni correction the MCI<sub>aTau</sub> group still had significantly lower FA ( $p = 0.03$ ) and higher DR ( $p = 0.042$ ) in the left posterior cingulum fibers compared to controls.

For the whole SCI/MCI group the mean T-tau value was 367 ng/L (SD: 266; range 79–1399). CSF T-tau values were negatively correlated with FA and positively correlated with DR in both right and left posterior cingulum fibers (right/left posterior cingulum Pearson's  $r$  for FA  $-0.37/-0.31$ ,  $p < 0.05$ , for DR 0.40/0.40,  $p < 0.01$ ), reaching significant levels also when regressing out age, sex, WMH volume, and MRI scanner (Table 3).

After excluding the eight patients diagnosed with SCI, the analysis were repeated with similar results. Only one of the SCI patients had elevated CSF T-tau, leaving 11 patients in the MCI group with pathological CSF T-tau values and 28 patients in the MCI group with normal CSF T-tau values. FA was significantly higher and DR was significantly lower in the MCI patients with pathologically elevated T-tau levels compared to controls (Supplementary Fig. 2).

#### 4. Discussion

CSF T-tau levels predict progression of SCI/MCI patients to dementia (Diniz et al., 2007; Hansson et al., 2006) and previous studies suggest that elevated levels of T-tau are markers for axonal damage (Sunderland et al., 2003; Sussmuth et al., 2001; Teunissen et al., 2005), to which DTI measures are expected to be sensitive. Thus it is of interest to determine which imaging parameters are associated with elevated T-tau levels. To our knowledge this is the first study testing the relationship between CSF T-tau values and white matter diffusivity in SCI/MCI patients. The main finding was that elevated CSF T-tau values were related to lower FA

and increased DR in these patients. Signs of decreased FA and increased DR in posterior cingulum fiber tracts of these patients indicate early tau-related white matter alterations. Increased DR may reflect loss of myelin (Pierpaoli et al., 2001; Song et al., 2002, 2003), but DR is a sensitive measure possibly also reflecting distant pathological processes (Budde et al., 2007). Our findings in the left posterior cingulum bundle are in line with previous studies of MCI patients (Fellgiebel et al., 2005; Zhang et al., 2007). The significant FA and DR alterations we observed in the genu of CC may indicate that early white matter pathology also appear in neocortical connections in patients at risk of developing AD.

Though we did not find group differences in DA, axon loss with transient effects on DA can not be excluded (Concha et al., 2006; Sun et al., 2008) and the observed association between DR and CSF T-tau levels may suggest underlying axonal affection (Teunissen et al., 2005). Biochemical studies of white matter (WM) have reported altered myelination in AD patients (Roher et al., 2002), which may be secondary to gray matter degeneration. However, WM degeneration has been found in early AD without cortical thinning (de la Monte, 1989), suggesting that WM is affected before cortical degeneration occurs. A recent study of AD patients by Salat et al. (2008), showed diffusion alterations in parahippocampal WM also after correcting for hippocampal volume. The authors suggest that besides cortical degeneration, WM degeneration may represent an additional mechanism in the AD process.

Tau pathology has been related to both axonal damage and hippocampal atrophy (Adalbert et al., 2007; de Leon et al., 2007). In a subsample of the present cohort, we recently reported associations between pathological CSF biomarkers and reduced hippocampal volume and cortical thinning in MCI patients (Fjell et al., 2008). We found no association between hippocampal volume and directional diffusivity in this sample (data not shown), though both were associated with CSF T-tau levels.

The present study has several limitations. The use of two different scanners with slight differences in acquisition matrices may possibly introduce biases, but the reported effects were observed in both samples and scanner site was regressed out in all analyses. Further, DTI acquisition with anisotropic

voxels could lead to an increase in partial volume effects with consequent under-estimation of FA and lack of sensitivity. Also, cognition in controls was not formally tested. However, neither of these limitations are likely to introduce a systematic bias in the results.

The present study was cross-sectional with a relatively small number of participants and the findings need to be confirmed in longitudinal studies with larger patient populations. However, also findings from functional imaging studies (Mosconi, 2005) as well as previous DTI studies (Fellgiebel et al., 2005; Zhang et al., 2007) suggest affection of the posterior cingulate area in MCI. Separate analysis of SCI and MCI patients should be done in larger patient populations. However, when we excluded the eight patients with SCI from the analysis we obtained the same results. If the transition between SCI and MCI is gradual, the use of CSF biomarkers and imaging parameters are essential to determine the underlying pathology. Follow-up studies are needed to confirm which of these patients will convert to AD. The focus of this study was to study putative associations between T-tau and DTI parameters. Patients with amnesic MCI may be more likely to progress to AD than patients with non-amnesic MCI (Petersen and Negash, 2008). Increased DR in the cingulum may well be a characteristic particularly of the patients with amnesic MCI, but this should be analysed in a larger patient population.

The present findings of increased radial diffusion in the posterior cingulum of SCI/MCI patients may be due to myelin loss, suggesting that either demyelination or reduced axonal integrity may be early features of the disease process. These changes may be part of a common pathway, related to memory network function and possibly occurring on the basis of early incipient Alzheimer-related pathology (Braak and Braak, 1997).

### Conflict of interest

The authors have reported no conflicts of interest.

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### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neurobiolaging.2009.04.014.

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