Depression severity is correlated to the integrity of white matter fiber tracts in late-onset major depression

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ABSTRACT

Cerebral white matter lesions (WMLs) are believed to play an important role in a subset of major depression (MD). We aimed to describe the impact of WMLs on white matter pathways in MD using diffusion tensor imaging (DTI) and magnetization transfer imaging. As a novel approach, we used DTI tractography to assess pathways intersected by WMLs. We examined 22 patients with late-onset MD and 22 age- and gender-matched controls. Parametric maps of fractional anisotropy (FA), apparent diffusion coefficient (ADC), and magnetization transfer ratio (MTR) were obtained to describe tissue integrity. The association between depression severity and the tract-specific localization of WMLs was analyzed on a voxel-by-voxel basis. We showed a significant positive association between depression severity and fiber tracts intersected by WMLs in the left superior longitudinal fasciculus and the right uncinate fasciculus. In both groups, WMLs had significantly lower FA and MTR, and higher ADC than both the tracts they intersected and the normal-appearing white matter (NAWM). In turn, the tracts intersected by WMLs had significantly lower FA and higher ADC than the NAWM. In conclusion, depression severity correlates with the tract-specific localization of WMLs. WMLs have a pronounced effect on white matter integrity in the pathways they intersect.

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1. Introduction

Imaging studies using T2-weighted magnetic resonance imaging (MRI) have reported an increased frequency of white matter hyperintensities, sometimes referred to as white matter lesions (WMLs), in major depression (MD), especially in late-onset and late-life MD (Herrmann et al., 2008; Videbech, 1997). As stated in the vascular depression hypothesis (Alexopoulos et al., 1997; Krishnan et al., 1997; Krishnan and McDonald, 1995), subcortical vascular changes may be an important contributor to WMLs, which in turn contribute to the pathogenesis in a subgroup of late-life MD by affecting mood-regulating neuronal pathways, either by single, localized lesions or by an accumulation of lesions exceeding a certain threshold (Alexopoulos et al., 1997). The presence of subcortical WMLs in late-life depression has been associated with cognitive impairment (Austin et al., 2001; Goodwin, 1997; Herrmann et al., 2007), functional impairment (Steffens et al., 2002), poor treatment outcome (Alexopoulos et al., 2002, 2008; Chen et al., 2006; Hickie et al., 1995, 1997; Iosifescu et al., 2006; O'Brien et al., 1998; Simpson et al., 1998; Steffens et al., 2001;

Taylor et al., 2003b), and a greater risk of subsequent dementia (Steffens et al., 2007). Although reports in the depression literature have found the WMLs to be mainly located in fronto-striatal circuits (Greenwald et al., 1998; MacFall et al., 2001; O'Brien et al., 2006; Sheline et al., 2008; Taylor et al., 2003a; Videbech et al., 2004), and in the basal ganglia (Videbech, 1997), the relation between lesion characteristics, their interference with specific white matter pathways, severity of symptoms, and disease remains largely unknown.

Recent advances in MRI technology, such as diffusion tensor imaging (DTI) and magnetization transfer imaging have facilitated the study of microstructural changes in neuropsychiatry. Magnetization transfer (MT) imaging is sensitive to water bound to macromolecules and has been demonstrated in post mortem studies to correlate with myelin content and axonal density (Chen et al., 2007; van Waesberghe et al., 1999). The method, quantified by the magnetization transfer ratio (MTR), which is a compound index of the exchange between free and protein-bound water protons pools, is regarded as superior to conventional MRI with respect to the detection and quantification of subtle white matter changes, e.g. in multiple sclerosis (Tofts et al., 2003). Studies using MT imaging in depression have revealed lower MTR in multiple white matter regions, including the fronto-striatal and limbic regions, and the genu and splenium of the corpus callosum (Gunning-Dixon et al., 2008; Kumar et al., 2004).

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DTI is based on the random thermal diffusion of water molecules (Basser et al., 1994; Pierpaoli et al., 1996) and is highly sensitive to changes in tissue microstructure, such as axonal injury (e.g. demyelination) and intracellular or extracellular edema, which may not be visualized with conventional MRI. Water diffusion is restricted by barriers such as cell membranes and myelin, which introduces a directional dependence termed *anisotropy*, quantified by means of an FA index (Beaulieu, 2009). The apparent diffusion coefficient (ADC) measures the magnitude of diffusion. DTI studies in MD have shown reduced FA in several white matter regions, primarily the prefrontal white matter regions, such as the superior frontal gyrus and anterior cingulate cortex (ACC), and in temporal regions, in elderly depressed subjects compared with controls (Bae et al., 2006; Nobuhara et al., 2006; Taylor et al., 2004; Yang et al., 2007). In general, DTI studies of affective disorders consistently identify reduced anisotropy in the frontal and temporal lobes and tracts of subjects with affective disorders relative to control subjects (Sexton et al., 2009). In remitted geriatric depression others have found the FA reductions to be more widespread and also to include the parietal and occipital regions as well as the basal ganglia (Yuan et al., 2007). In addition, changes in FA in distributed cerebral networks have been associated with failure to remit in geriatric depression (Alexopoulos et al., 2008; Taylor et al., 2008).

During the last decade, advances in DTI have facilitated the study of tract-specific measures of cerebral microstructural integrity *in vivo*, a technique known as tractography or fiber tracking (Basser et al., 2000) – see, for example, the review by Mori and van Zijl (2002). DTI tractography combines measurements of water diffusion direction in each image voxel to reconstruct the trajectory of fiber pathways, thus reflecting neural connectivity (Basser et al., 2000). The method has enabled virtual “dissections” of major white matter fascicles in the living brain (e.g., see Catani et al., 2002). Application of DTI tractography in depression has mainly been to define and describe anatomical connectivity in well-defined regions of interest, such as targets of deep brain stimulation (Gutman et al., 2009; Johansen-Berg et al., 2008).

We have previously reported on WMLs and vascular risk factors in late-onset MD (Dalby et al., 2010). In this study, we present a novel approach to examine the impact of WMLs on gray matter connectivity by using DTI tractography to reveal the pathways potentially affected by WMLs. To our knowledge, DTI tractography has not previously been implemented to assess the anatomical extent and impact of WMLs on white matter integrity, which we present here. The aim of our study was to combine tractography and measures of white matter integrity to determine the tract-specific localization of WMLs, and to characterize the WMLs as well as the tracts they intersect in patients with late-onset MD compared with non-depressed, age- and gender-matched controls. Specifically, we hypothesized that in late-onset MD: 1) WMLs co-locate with mood-regulating pathways to a higher extent in patients than in non-depressed controls, that 2) depression severity is associated with WMLs intersecting mood-regulating pathways, that 3) WMLs show impaired white matter integrity compared with the surrounding white matter, and that 4) fiber tracts intersected by WMLs show impaired white matter integrity compared with the surrounding white matter.

2. Materials and methods

2.1. Subjects

Whole-brain MRI data were acquired from 22 patients with late-onset, first-episode MD and 22 controls with no history of psychiatric illness. The two groups were matched for age and gender. A brief account of the clinical and socio-demographic data is given in Table 1. The patients were consecutively recruited from psychiatric hospitals in the County of Aarhus through referral to the Neuropsychiatric

Table 1

Socio-demographic and clinical characteristics of patients with late-onset major depression and non-depressed controls. MES = Bech–Rafaelsen Melancholia Scale.

	Patients (n = 22)		Controls (n = 22)		Statistics	
	Mean	S.D.	Mean	S.D.	z	P
Age (years)	57.4	4.6	59.2	7.3	0.5	0.64
Vascular risk factor score ^a	6.9	4.7	6.8	4.4	0.05	0.96
MES score	16.5	5.8	0.3	0.8	−5.9	<0.001 ^b
	n	%	n	%		P
Gender						1.00
Male	7	31.8	7	31.8	–	–
Female	15	68.2	15	68.2	–	–

^a Composite vascular risk factor score as described in the Framingham Study (Wolf et al., 1991), comprising age, systolic blood pressure, antihypertensive treatment, diabetes, cigarette smoking, cardiovascular disease (coronary heart disease, cardiac failure, or intermittent claudication), atrial fibrillation, and left ventricular hypertrophy.

^b Significant results at $P < 0.05$.

Clinic, Aarhus University Hospital, Risskov, Denmark, and from outpatient psychiatric clinics in the County of Aarhus, Denmark. All patients met the DSM-IV (American Psychiatric Association, 2000) criteria for major depression and the ICD-10 criteria (World Health Organization, 1993) for moderate to severe depression within 4 weeks of inclusion. Late onset was predefined as first onset of depressive symptoms after the age of 50 years. Controls were recruited through advertisement in local papers and went through a preliminary sorting for exclusion criteria (see below) in a thorough telephone screening procedure. At the inclusion all subjects were assessed with selected parts of the SCAN structured interview (Wing et al., 1998) and were rated for severity of depressive symptoms with the Bech–Rafaelsen Melancholia Scale (MES) (Bech, 2002), which is an extended version of the 6-item Hamilton Depression Scale. The patients underwent a clinical neuropsychological examination or alternatively the Mini-Mental State Examination (MMSE) test (Folstein et al., 1975), depending on clinical and practical circumstances (results not shown). All controls were screened with the MMSE test. Both patients and controls were thoroughly interviewed about their medical history and were screened for concurrent medical diseases and alcohol abuse by standard blood tests, including thyroid function, and they all underwent a neurological exam. According to the Edinburgh Handedness Inventory (Oldfield, 1971), all included subjects were right-handed, except for three patients and one control who were ambidextrous. Current medication and information on vascular risk factors, such as hypertension and smoking, was carefully recorded (Dalby et al., 2010), and a composite vascular risk score was calculated as defined in the Framingham Study (Wolf et al., 1991). Exclusion criteria for both groups were organic brain disease (e.g. former stroke, cerebral vascular malformations, or epilepsy), former brain injury, substance dependency, and conventional contraindications to undergo MRI scanning. Written informed consent was obtained from all study subjects, and the study was approved by the regional ethics committee on research and in accordance with the Declaration of Helsinki (World Medical Association, 2008).

2.2. Scan protocol

MRI scans were obtained with a whole-body 3T GE Signa HDx scanner (GE Medical Systems, Milwaukee, WI, USA). The MRI protocol consisted of an axial fast spoiled gradient echo (FSPGR) 3D T1-weighted sequence (TE = 2.84 ms, TR = 6.64 ms, TI = 750 ms, flip angle = 14°, field of view (FOV) = 240 mm, matrix 256 × 256, slice thickness = 1.2 mm, no gap), an axial T2-weighted fluid-attenuated inversion recovery (FLAIR) sequence (TE = 120 ms, TR = 8650 ms, TI = 2250 ms, FOV = 240 mm, matrix 224 × 256, slice thickness = 5 mm, gap = 1.5 mm), an axial T2-weighted sequence (TE = 112 ms, TR = 5400 ms, field of view =

240 mm, matrix 416×416 , slice thickness = 5 mm, gap = 1.5 mm), and an axial DTI sequence (TE = 79.7 ms, TR = 12500 ms, flip angle = 14, FOV = 240 mm, matrix 128×128 , slice thickness = 3.5 mm, no gap, acquisition time approximately 7 min). The DTI scan was performed using double spin echo single shot EPI with 26 gradient directions ($b = 1000 \text{ s/mm}^2$) and 6 $b = 0 \text{ s/mm}^2$ acquisitions. The MT protocol consisted of two axial FSPGR 3D sequences (TE = 6.9 ms, TR = 33 ms, flip angle = 5, FOV = 240 mm, acquisition matrix = 256×160 interpolated to 256×256 , slice thickness = 2.0 mm); one with a MT saturation pulse and one without. The MT pulse was a Fermi-shaped pre-pulse with a nominal flip angle of 1100° , 8 ms duration and 2400 Hz offset. The whole-body RF coil was used for transmission and is believed to be sufficiently homogenous for this application. We used comfortable, stabilizing foam pads to minimize subject head motion. Total acquisition time was approximately 50 min.

2.3. Image analysis

The T1-weighted images were non-linearly transformed to match the MNI standard brain (Evans et al., 1994; Mazziotta et al., 2001). The subject-to-MNI space transformation was estimated using the ANIMAL algorithm (Collins et al., 1995; Robbins et al., 2004).

Diffusion tensors, FA, and ADC were calculated in each voxel of the brain according to the method of Le Bihan et al. (2001). MTR was calculated on a voxel-by-voxel basis as:

$$MTR = \frac{M_0 - M_s}{M_0},$$

where M_s and M_0 represent signal intensities with and without the saturation pulse (Henkelman et al., 2001). The FA, ADC, and MTR data were co-registered with the T1 data in native space and were normalized to standard MNI space using the non-linear T1-to-MNI transformation, estimated using the ANIMAL algorithm, with a final voxel size of 1 mm^3 .

2.4. White matter lesions

In order to reduce the possible neuropathological heterogeneity in the lesion pool, we omitted periventricular WMLs from assessment and only included subcortical/deep WMLs (DWMLs). This decision was based on previous studies reporting distinct neuropathological (Fazekas et al., 1993; Thomas et al., 2002, 2003) as well as functional differences (Kim et al., 2008) between DWMLs and PVLs. In addition, the existing literature indicates a stronger association between DWMLs and depression (Krishnan et al., 2006), especially late-onset MD (O'Brien et al., 1996), compared with PVLs.

The DWMLs were identified as hyperintensities on the FLAIR images by an experienced neuroradiologist, blinded to subject status. Intra-rater reliability testing was not carried out. The T1- and T2-weighted images were available for differential-diagnostic purposes. The identified lesions were manually labeled on the FLAIR images using Display software (McConnell Brain Imaging Centre, MNI, McGill University, Montreal, Quebec, Canada). The labeled lesions for each subject were resampled to a binary lesion mask in native space, each voxel presenting a value of 0 (= no lesion) or 1 (= lesion). An average map of lesion density based on the lesion masks in the patients and controls has been previously published (Dalby et al., 2010).

2.5. Tractography procedure

Tractography was performed in native space using in-house implementation of the *fiber assignment with continuous tracking* (FACT) algorithm as described by Mori et al. (Mori et al., 1999; Mori and van Zijl, 2002) with standard stopping criteria (FA < 0.15 and angle between neighboring primary diffusion direction < 45°). The in-

house developed software for visualization and tractography has been previously applied (Moller et al., 2007). Details on DTI acquisition parameters are given above. Tracking was initiated from the center of every voxel in the brain. As stated above, the binary image masks identifying DWMLs (lesion masks) were manually derived by labeling intensity hyperintensities on FLAIR image volumes for each subject. By applying the binary lesion masks to our tractography results from the DTI data, we identified the fiber tracts intersecting one or more DWMLs. The voxels for each of these fiber tracts were then retained as a separate image mask (secondary tractography masks). The resulting secondary tracking mask for each individual was saved as a binary mask, each voxel assigned a value of 0 (= no tract) or 1 (= tract intersecting one or more DWMLs). An illustration of the main steps in the tracking approach is given in Fig. 1. Before applying the binary lesion masks to our tractography results, each lesion was dilated one voxel in each direction to include the possible partial volume effects of border voxels.

The Supplementary Figs. SF1 and SF1 give an average map of the secondary tractography masks (i.e. the density of fiber tracts intersecting DWMLs) for the patients and controls, respectively. Selected slices of these average maps are presented in Fig. 2A and B.

For the voxel-based analyses, all individual secondary tracking masks were transformed to MNI standard space using the MRI-to-MNI space transformation, estimated using the T1-weighted data. These masks were subsequently blurred with a 4 mm full-width-half-maximum Gaussian kernel to decrease spatial noise and smooth the data.

2.6. Tissue characterization

Classification of the MRI volume into gray and white matter was estimated from the T1-weighted images by using a neural network-based approach (Cocosco et al., 2003). To avoid the effect of WMLs on white matter characteristics, an individual mask of normal-appearing white matter (NAWM) was made by subtracting the segmented DWMLs from the initial white matter mask in each subject and subsequently eroding the NAWM mask by one voxel in each direction to avoid partial volume effects with the WMLs, surrounding gray matter, and ventricles.

Median values of ADC, FA, and MTR were extracted from each individual's corresponding parametric maps in native space using the following ROIs: a) Lesion masks (DWMLs), b) secondary tracking masks, and c) NAWM masks. Prior to extraction, the DWMLs were also excluded from the secondary tracking masks to avoid the effect of lesions on tract characteristics.

Median values were chosen over mean values, as medians are less sensitive to extreme scores and are more appropriate for smaller sample sizes.

2.7. Statistical analyses

The median values of ADC, FA, and MTR, in each ROI were compared between groups with the Wilcoxon two-sample rank sum test (Mann–Whitney test) for continuous variables using Stata, release 9.2 (Stata Statistical Software, StataCorp LP, College Station, Texas, USA) with two-sided tests and a 5% level of significance. To compare ADC, FA, and MTR between regions, e.g. median FA in DWMLs compared with median FA in whole-brain NAWM, we used the Wilcoxon signed-rank sum test, which is a non-parametric counterpart to the paired *t*-test.

We used FMRISTAT (Worsley et al., 2002) to test for voxel-wise differences in ADC, FA, and MTR values between groups and to test for differences in the localization of tracts intersected by WMLs between patients and controls with one or more lesions, adjusted for age and gender. The effect of MES score on tractography outcome was studied using voxel-wise regression analyses. Resulting *t*-maps were examined

Fig. 1. Illustration of the main steps in the tractography procedure involving WMLs. 1. Deep WMLs (DWMLs) were manually labeled (red) in each subject; in this subject only two DWMLs. The labeled lesions for each subject were resampled to a binary lesion mask in native space, here superimposed on the subject's own T1-weighted dataset, each voxel presenting a value of 0 (=no lesion) or 1 (=lesion). 2. Tractography was initiated from the center of all voxels in the brain. By applying the binary lesion masks to our tractography results we identified the fiber tracts (green) intersecting one or more DWMLs (red). The voxels for each of these fiber tracts were then retained as a separate image mask (secondary tractography masks). Before applying the binary lesion masks to our tractography results, each lesion was dilated one voxel in each direction to include the possible partial volume effects of border voxels. 3. The resulting secondary tracking mask for each individual was saved as a binary mask, each voxel assigned a value of 0 (=no tract) or 1 (=tract intersecting one or more DWMLs). 4. The final binary tracking mask, here representing fiber tracts intersecting two DWMLs, superimposed on the subject's own T1-weighted data set.

for significant changes, including summary analyses of *t*-statistic images and correction for multiple comparisons, based on the 3D Gaussian random field theory (Worsley et al., 1996). We used the method of Cao (1999) to identify significant clusters, defined as a minimum number of 26-connected voxels at $t < -3.17$ or $t > 3.17$ ($P < 0.05$). The resultant images were reviewed in Register (McConnell Brain Imaging Centre, MNI, McGill University, Montreal, Quebec, Canada), and the white matter tracts were anatomically evaluated according to the DTI-based white matter atlas by Mori et al. (2005).

3. Results

Patients and controls showed a similar composite vascular risk factor score (Table 1). Details on vascular risk factors and lesion load have been previously described (Dalby et al., 2010). We reported no significant difference in the number [$P = 0.63$] or volume [$P = 0.65$] of DWMLs between groups (data not shown).

3.1. Tractography

Overall, voxel-based analyses did not reveal any significant difference in the localization of fiber tracts intersected by DWMLs patients and controls [$P = 0.37$], adjusted for age and gender.

An average map of the secondary tracking masks in standard space was made for the patients and controls, respectively, to illustrate the distribution of trajectories affected by WMLs in the two groups (see Supplementary Figs. SF1 and SF2 for a full overview). Selected slices of these average maps are shown in the left panel of Fig. 2A and B. The

slices were selected as they featured the areas with the greatest difference in the localization of tracts intersected by WMLs between groups. This difference is pointed out in the subtraction map in the adjacent right panel, which shows where more patients than controls had tracts intersected by WMLs. The subtraction map shows that both the frontal projections of the genu of the corpus callosum (Fig. 2A), as well as the opercular part of the right superior longitudinal fasciculus (SLF) (Fig. 2B), were intersected in up to 30% more patients than controls. However, these apparent differences in localization did not reach statistical significance.

Post-hoc voxel-based analyses for depression severity (MES score) were only carried out for the patient group ($n = 22$), as the controls – as expected – scored 0 or very low on this scale (see Table 1). A significant positive association between higher MES score and secondary tractography masks, adjusted for age and gender, was found in two areas, as illustrated in Fig. 3A, the left operculum corresponding to the opercular part of the left SLF [$P = 0.03$], and B) the right superior temporal gyrus corresponding to the temporal projections of the right uncinate fasciculus [$P = 0.04$].

3.2. Tissue characterization

Voxel-based analysis did not reveal any significant areas with lower FA [$P = 0.83$], higher ADC [$P = 0.90$], or lower MTR [$P = 0.94$] in patients compared with controls.

Non-parametric testing of differences in median values of FA, ADC, and MTR in a) lesion masks (DWMLs), b) secondary tracking masks, and c) NAWM masks, respectively, showed no significant differences

Fig. 2. A and B illustration of two areas, A (upper) and B (lower), with marked differences in the localization of tracts intersected by DWMLs between patients and controls with one or more DWMLs. *Left panel:* An average map of the tracking masks in standard space was made for the patients and controls, respectively, with one or more DWMLs to illustrate the overall distribution of tracts intersecting DWMLs in the two groups. *Right panel, left:* A subtraction map showing the percent-wise difference between tracts intersected by DWMLs in patients versus controls. The subtraction map represents the areas where more patients than controls have tracts intersected by DWMLs. The subtraction map in A shows that the frontal projections of the genu of corpus callosum, also known as the anterior forceps (forceps minor), was affected in up to 30% more patients than controls, while the subtraction map in B shows that the opercular part of the right superior longitudinal fasciculus was affected in up to 30% more patients than controls. *Right panel, right:* T-maps resulting from voxel-based *t*-testing of differences in the tract localization between patients and controls. The highest T-values are seen in the frontal projections of the genu of the corpus callosum (A) and in the opercular section of the right superior longitudinal fasciculus (B), but did not reach statistical significance ($P > 0.05$). DWMLs = deep white matter lesions.

between groups; results are summarized in Table 2. Because of the similar characteristics, the two groups were pooled for succeeding analyses. The right section of Table 2 shows the results from analyses of the pooled regional FA, ADC, and MTR for all subjects with one or more DWMLs regardless of subject status ($n = 38$). Paired non-parametric

testing showed that DWMLs had significantly lower FA and MTR, and higher ADC than both the tracts they intersected and the whole-brain NAWM. In turn, the tracts intersected by DWMLs had significantly lower FA and higher ADC compared with the whole-brain NAWM; there was a trend towards significantly lower MTR [$P = 0.10$].

Fig. 3. Illustration of the clusters with a significant positive association with MES score for all patients ($n = 22$), adjusted for age and gender. *Left panel:* The clusters (red) represent tracts intersected by DWMLs and are situated in the opercular part of the left SLF (A) and in the right superior temporal gyrus (B), the latter corresponding to the temporal projections of the right uncinate fasciculus. *Right panel:* The corresponding T -maps. MES = Bech–Rafaelson Melancholia Scale. DWMLs = deep white matter lesions. SLF = superior longitudinal fasciculus.

4. Discussion

In this study we combined an innovative DTI tractography approach with standard diffusion and magnetization transfer measures to assess the anatomical extent and impact of DWMLs on white matter fiber pathways in patients with late-onset MD compared with non-depressed, age- and gender-matched controls. The principal finding of our study is that DWMLs profoundly affect white matter integrity, measured by diffusion and magnetization transfer parameters, both within the lesion sites themselves and along the neuronal pathways they intersect, and do so in a similar manner in patients and controls. Although the overall tract-specific localization of WMLs did not differ between patients and controls, we showed that

depression severity correlates with fiber tracts intersected by DWMLs in pathways involved in the regulation of mood and cognition.

4.1. Tissue characterization

While our measurements did not permit us to directly address the underlying pathoanatomical damage to fiber architecture, previous studies correlating MRI findings to post mortem studies suggest that WMLs may indeed cause widespread degenerative changes in both normal aging (Awad et al., 1986a; Fazekas et al., 2005) and disease (Schmierer et al., 2004; van Waesberghe et al., 1999). Neuropathological correlates of DWMLs are diverse and include arteriosclerosis, atrophic demyelination, enlarged perivascular spaces, and vascular

Table 2

Diffusion and magnetization transfer characteristics of DWMLs, tracts intersected by DWMLs, and NAWM in patients with late-onset major depression and non-depressed controls.

	All subjects (<i>n</i> = 44)						Subjects with WMLs (<i>n</i> = 38)		
	Patients (<i>n</i> = 22)		Controls (<i>n</i> = 22)		Statistics		Comparison	Statistics	
	Median	95% CI ^a	Median	95% CI ^a	<i>z</i>	<i>P</i>		<i>z</i>	<i>P</i>
<i>FA</i>									
a) DWMLs ^b	0.33	0.32–0.34	0.33	0.31–0.36	0.40	0.69	a versus b	− 5.26	<0.001
b) Tracts ^b	0.40	0.39–0.42	0.42	0.41–0.44	1.07	0.28	a versus c	− 5.33	<0.001
c) NAWM	0.43	0.42–0.44	0.43	0.42–0.44	0.21	0.83	b versus c	− 3.67	<0.001
<i>ADC</i> (<i>10</i> ^{−3} <i>mm</i> ² / <i>s</i>)									
a) DWMLs ^b	0.79	0.78–0.82	0.83	0.80–0.85	1.42	0.15	a versus b	5.36	<0.001
b) Tracts ^b	0.74	0.71–0.77	0.75	0.73–0.77	0.07	0.94	a versus c	5.36	<0.001
c) NAWM	0.69	0.67–0.72	0.73	0.71–0.75	0.78	0.44	b versus c	4.87	<0.001
<i>MTR</i>									
a) DWMLs ^b	0.66	0.63–0.69	0.62	0.60–0.63	− 1.57	0.12	a versus b	− 4.31	<0.001
b) Tracts ^b	0.68	0.65–0.70	0.63	0.60–0.65	− 0.95	0.34	a versus c	− 4.57	<0.001
c) NAWM	0.68	0.65–0.70	0.63	0.61–0.66	− 1.55	0.12	b versus c	− 1.67	0.10

Lesion volume of DWMLs was excluded from tracts and whole-brain NAWM volume before analysis. *Left section:* The statistics column shows the results from non-parametric testing of median values of FA, ADC, and MTR between patients and controls in three ROIs: DWMLs, tract intersected by DWMLs, and whole-brain NAWM. *Right section:* The statistics column shows the results from paired non-parametric testing of median values of FA, ADC, and MTR, in DWMLs and white matter tracts intersected by DWMLs, respectively, versus corresponding values in whole-brain NAWM. DWMLs = deep white matter lesions. FA = fractional anisotropy. ADC = apparent diffusion coefficient. MTR = magnetization transfer ratio. NAWM = normal-appearing white matter.

^a Approximated 95% confidence interval (CI) after McGill et al. (1978).

^b Only subjects with WMLs (17 patients and 21 controls).

ectasia (Theobald et al., 2000). Microvascular disturbances ultimately resulting in cerebral ischemia have been proposed as playing a major role in the pathogenesis of WMLs. However, the causes of WMLs are incompletely understood, and may be the result of several mechanisms resulting in local increased brain water content as reflected in the T2-weighted MRI images (Pantoni and Garcia, 1995). The presence and severity of WMLs increase with age and have been shown to correlate with vascular risk factors, such as hypertension and smoking (de Leeuw et al., 2002; Longstreth et al., 2005). In agreement with previous findings by Taylor et al. (2001), we were not able to demonstrate any difference between patients and controls in diffusion characteristics of the DWMLs, and this finding recurred in the tracts intersected by DWMLs. The same was applicable for the MTR, in support of a similar origin of lesions in patients and controls, which may ultimately reflect the equal vascular risk factor score between groups (Table 1).

A novel finding is the observed significant changes in diffusion parameters in the fiber tracts intersected by DWMLs, compared with the DWMLs themselves and the surrounding NAWM in both groups. Although previous studies of late-life MD have reported microstructural abnormalities in NAWM in depressed patients compared with healthy controls (Kumar et al., 2004), even after excluding visible WMLs (Shimony et al., 2009), no previous literature has described the tract-specific impact of the WMLs on brain microstructure *in vivo* as presented here. In comparison, the observation of changes in ADC, FA, and MTR in DWMLs is in agreement with several previous studies of late-life depression (Fazekas et al., 2005; Filippi, 2001; Gouw et al., 2008; Shimony et al., 2009; Tanabe et al., 1999; Taylor et al., 2001). Due to the relative changes between DWMLs, the tracts they intersect, and the NAWM, our results suggest a pronounced effect of DWMLs on white matter integrity, most profoundly localized to the lesion sites themselves, but also – albeit to a lesser extent – in the tracts they intersect. However, the cross-sectional design of our study does not allow us to conclude on any causal link between the DWMLs and the observed changes in white matter integrity of the tracts they intersect. We have previously reported smoking as a major contributor to the number and volume of DWMLs (Dalby et al., 2010). Smoking is considered a risk factor for WMLs, possibly on the basis of long-term damaging effects attributed to atherosclerosis and oxidative stress, mediated by inflammatory processes and nicotine-induced free radicals (Jane-Llopis and Matysina, 2006). Cigarette smoking has been shown to be an independent predictor of worsening white matter grade in the elderly (Longstreth et al., 2005). However, a recent study suggested that healthy adults with low exposure to cigarette smoking showed increased microstructural integrity of the white matter compared with either no or high exposure (Paul et al., 2008).

Surprisingly, our patients with late-onset MD did not show greater microstructural abnormalities, measured as voxel-wise changes in FA, ADC, or MTR, in any region, including the fronto-striatal regions, compared with the controls. This is in contrast to previous findings (Bae et al., 2006; Gunning-Dixon et al., 2008; Nobuhara et al., 2006; Taylor et al., 2004). Several explanations apply to our negative findings; firstly, our choice of voxel-based analysis generally carry lower statistical power than ROI analyses, which are used in most previous studies (Bae et al., 2006; Nobuhara et al., 2006; Taylor et al., 2004). Secondly, our sample size was lower than in two of the mentioned studies (Bae et al., 2006; Gunning-Dixon et al., 2008), thereby introducing the risk of type 2 errors. However, in these previous studies no – or only sparse – information on the concurrent presence and distribution of WMLs and vascular risk factors across groups were provided. As increased WMLs volumes have been associated with lower FA and higher ADC values, indicative of widespread effects on microstructural integrity (Taylor et al., 2007), and as WMLs are generally associated with the presence of vascular risk factors (Awad et al., 1986b; Breteler et al., 1994; Fazekas et al.,

1993; Liao et al., 1997), we therefore speculate that our negative findings may be a consequence of an equal lesion load and similar composite vascular risk factor scores between groups (Dalby et al., 2010).

4.2. Fiber tract localization

Interestingly, we were not able to show any significant difference in the overall localization of fiber tracts intersected by DWMLs in patients compared with controls, which contradicts our first hypothesis, based on previous literature on the localization of WMLs (Herrmann et al., 2008; Videbech, 1997). The main reason for this inconsistency could be the limited power of our study due to a relatively small sample size, thus introducing the risk of a type 2 error. The age of our sample is lower than in most previous studies of late-onset MD. We chose a lower cut-off at 50 years to pass the female menopause. On the one hand, this may be a strength to our study, thereby including relatively younger patients with different degrees of vascular risk factors. On the other hand, a relatively younger sample may dilute isolated findings in an older population. In addition, while findings in previous studies may reflect the impact on brain microstructure of recurrent episodes, we chose to include only patients with first-episode MD in order to reduce heterogeneity in the course of illness. Furthermore, our patients and controls had a similar lesion load and a similar vascular risk factor score, which improves the comparability between the two groups. The similar vascular risk factor score between groups may reflect the intentional inclusion of controls presenting with vascular risk factors, such as hypertension, in order to match the vascular profile of our patient group. Finally, our tractography findings emphasize the fact that WMLs may affect a given pathway anywhere along its course, i.e. disruption of a pathway is not necessarily a result of primary damage to a specific brain region, which makes it difficult to directly compare findings from tractography and lesion studies.

4.3. Depression severity

In the patients with late-onset MD we showed a significant positive association between depression severity (higher MES score) and having tracts intersected by DWMLs in the opercular part of the left SLF and the temporal projections of the right uncinate fasciculus (Fig. 3). These tract localizations are coincident with our group's previous findings regarding the localization of DWMLs in late-onset MD (Dalby et al., 2010). In general, DWMLs have been associated with severity of depression, severity of cognitive impairment, risk and outcome in MD (Kim et al., 2008). For example, MacFall et al. (2001) have found that severity of late-life depression was correlated with lesions in the medial orbital region. The uncinate fasciculus is considered part of the limbic system and is attributed to the functional aspects of emotion, behavior, and memory (Drevets et al., 2008). Our findings thus suggest an important role for the uncinate fasciculus in MD, in consistency with our second hypothesis. The SLF is a long association fiber bundle interconnecting areas within the frontal, parietal, occipital, and temporal lobes (Catani et al., 2002; Heimer, 1995; Makris et al., 2005). The human SLF can be segmented into 4 subregions: SLF I, II, III, and the arcuate fasciculus, which in turn play an important role in a variety of neurological or cognitive functions such as working memory, somatosensory responses, spatial attention, language, and motor behavior/function (Makris et al., 2005). Many of these functions are compromised to various degrees in late-life depression (Butters et al., 2004; Sheline et al., 2006). Some of the functions are lateralized, such as the attention to sensory stimuli which in humans primarily activates the prefrontal and superior parietal cortex in the right hemisphere (Pardo et al., 1991). Newer research indicates a potential role for the SLF in volition (Haggard, 2008), which is often impaired in MD. Indeed, the disturbances in

mood and cognitive functions in MD may result from a relative imbalance among several affected connections rather than disruption of a single pathway (Mayberg, 1997), in accordance with the vascular depression hypothesis (Alexopoulos et al., 1997). Thus, our findings imply an important role for tract-specific localization of DWMLs on the severity of symptoms in late-onset MD rather than a categorization of patients or controls. The clinical implications of these findings are intriguing, as our tractography approach provides more exact anatomical information of the tracts and circuitries affected by WMLs.

The clinical relevance of correlations between state variables such as depression severity and trait variables such as structural findings, including WMLs, is a matter of debate. Clinical variables often fluctuate over shorter time intervals than structural changes and may in basic terms be difficult to correlate. We acknowledge that it may be difficult to correlate observed symptoms with underlying anatomy. In our study, the patients were moderately to severely depressed, and the severity justified admission to a psychiatric hospital or out-patient psychiatric clinic. We assumed that rapid changes in MES score for these patients shortly after referral and inclusion would be unlikely. Our patients have an average MES score equaling a moderate depression (Table 1), declining towards recovery. Since our study design was cross-sectional, and the patients might have had a higher MES score (i.e. more severe depression) at a previous point before referral, this would only increase the significance and thereby strengthen the association between higher MES score and tractography findings. Medication is another confounder which may over time confuse depression severity rating with treatment response rates. Unfortunately, unmedicated patients were for practical and ethical reasons difficult to attain in our combined in- and out-patient setting.

4.4. Methodological issues

Our tractography approach raises some important methodological issues, first of all the question of whether tracking through WMLs is reasonable. Several studies have emphasized the heterogeneity of WMLs (Barkhof and Scheltens, 2002; Gouw et al., 2008; Spilt et al., 2006). The neuropathological correlates of WMLs are diverse and depend on lesion severity (Fazekas et al., 1993). Consequently, WMLs may affect tracts in various degrees, from minimal structural damage to total disconnection; if the latter was true in our study population, the substantial load of WMLs in our patients and controls would cause massive neurological symptoms, which was not the case. In addition, the tracking algorithm employed the standard stopping criteria of $FA < 15$ which is well below the measured median FA value of the DWMLs (Table 2). However, we cannot preclude that some lesions may totally disconnect a tract, or that localized edema may change the course of a tract, thereby terminating or altering the tracking in this area, and consequently underestimate the generated tracts. To account for this possibility our tractography protocol included the step of adding a voxel in each direction around a given lesion prior to the tracking, in the presumption that less affected surrounding tracts would be tracked instead. We used a deterministic tracking method, and application of voxel-based analysis to this kind of tractography data does not account for the spatial dependency in the tractography procedures, i.e. the likelihood of a voxel neighboring a tracked voxel also being tracked may be greater than for a voxel far away from this area. Accounting for this co-dependency calls for a future probabilistic approach. Nonetheless, voxel-based analysis is useful in exploratory approaches, where white matter changes are diffuse (Catani, 2006).

There are some limitations of our study, mainly related to the use of DTI tractography (Assaf and Pasternak, 2008). DTI acquisition parameters were chosen to accommodate both appropriate signal-to-noise ratio (SNR) and clinically acceptable total scan time. An isotropic “gold standard” spatial resolution (e.g. $2.5 \times 2.5 \times 2.5 \text{ mm}^3$) was not possible in our scan protocol ($1.88 \times 1.88 \times 3.5 \text{ mm}^3$), but the resulting

voxel size in our sample was actually smaller than in the isotropic example (Jones et al., 2002). We used 26 gradient directions for DTI, and according to Jones (2004) the advantage in reliable determination of tensor direction after 25–30 directions is minimal. Tractography is most sensitive to major fiber bundles, while small or tortuous paths are more challenging to track, making the method susceptible to false negatives. In addition, a pitfall of the diffusion tractography is that the calculated trajectory of the fiber may fail to follow the true fiber tract trajectory because of small uncertainties in the determination of the primary diffusion direction due to inherently noisy DTI, crossing fibers (Basser et al., 2000; Le Bihan, 2003), and partial volume effects (Alexander et al., 2001). Also, the tracking procedure itself may influence the results, for example regarding the choice of seed voxels. In our approach, tracking was initiated from all voxels within the brain, and only tracking results that penetrated the voxels of interest (i.e. the DWMLs) were kept. This is opposed to approaches mainly based on anatomically well-defined tracts which may impose limitations on the discovery on new or less explored tracts (Mori and van Zijl, 2002).

We chose to compare tract and lesion characteristics with NAWM. Indeed, the term NAWM should be used with caution, as MRI characteristics vary in different brain regions and in different acquisition protocols, depending on regional fiber orientation and organization. For example, highly structured white matter areas such as the long association fibers and commissural bundles would per definition have higher FA and MTR values than less organized white matter areas or white matter in areas with many crossing fibers, and the impact of WMLs may therefore vary relatively to the anatomical localization. Consequently, perfectly matching white matter areas for comparison analyses are very difficult to attain. In this study tracking was initiated from all voxels within the brain. Thus, our approach did not allow the usual ROI approach with pre-chosen start and end points to outline major fiber tracts. Therefore we were not able to define anatomical “control tracts”, but chose the NAWM for comparison instead. Importantly, as we segmented only DWMLs, thereby permitting PVLs in the NAWM masks, we cannot preclude a possible effect of PVLs on whole-brain white matter characteristics. However, as literature reports decreased FA and increased ADC in such PVLs (Bastin et al., 2009), this only strengthens our findings of reduced FA and ADC in the segmented DWMLs and intersecting pathways compared with the NAWM.

We used a manual segmentation method for the WMLs, which allowed us to distinguish between DWMLs and PVLs. While several (semi)automated techniques exist for the classification of WMLs, many of these techniques are not yet optimal (Kim et al., 2008) and their applicability to new datasets from different scanner models are unclear. The multiple sclerosis imaging community have developed advanced techniques with respect to the classification of WMLs, but most of the algorithms used integrate prior statistical knowledge based on the expected spatial distribution of these lesions, which would not hold for the classification of WMLs in MD. The T2-weighted FLAIR sequence used for identification and segmentation of WMLs had a slice thickness of 5 mm and a 1.5-mm gap between slices, thereby introducing the risk of sampling errors with respect to the definition of WMLs. However, these parameters were consistent through the data, and the slice thickness and gap were adopted to increase SNR, diminish cross-talk, and keep an acceptable acquisition time. A major strength of our study is the use of 3 T MRI, which allows better spatial resolution and increased SNR than 1.5 T MRI, thereby improving the detection of more subtle white matter changes.

In conclusion, we here present a novel approach using DTI tractography to describe the localization and impact of DWMLs on gray matter connectivity in late-onset major depression and non-depressed controls. Our results suggest that DWMLs have a marked effect on measures of white matter integrity both locally within the lesion site and along the neuronal pathways they intersect. Although

no overall difference in the tract-specific localization of WMLs was found between groups, depression severity correlated significantly with fiber tracts affected by DWMLs in pathways involved in mood and cognition. The use of DTI tractography as a segmentation tool for mapping cerebral pathways affected by WMLs allows future integrative approaches, combining cognitive and microstructural measures to address the functional impact of WMLs on tissue integrity in neuropsychiatric disease.

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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.psychres.2010.06.008](https://doi.org/10.1016/j.psychres.2010.06.008).

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