Localization of white-matter lesions and effect of vascular risk factors in late-onset major depression

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Background. Several studies suggest that patients with late-onset major depression (MD) have an increased load of cerebral white-matter lesions (WMLs) compared with age-matched controls. Vascular risk factors such as hypertension and smoking may confound such findings. Our aim was to investigate the association between the localization and load of WMLs in late-onset MD with respect to vascular risk factors.

Method. We examined 22 consecutive patients with late-onset first-episode MD and 22 age- and gender-matched controls using whole-brain magnetic resonance imaging (MRI). The localization, number and volume of WMLs were compared between patients and controls, while testing the effect of vascular risk factors.

Results. Among subjects with one or more WMLs, patients displayed a significantly higher WML density in two white-matter tracts: the left superior longitudinal fasciculus and the right frontal projections of the corpus callosum. These tracts are part of circuitries essential for cognitive and emotional functions. Analyses revealed no significant difference in the total number and volume of WMLs between groups. Patients and controls showed no difference in vascular risk factors, except for smoking. Lesion load was highly correlated with smoking.

Conclusions. Our results indicate that lesion localization rather than lesion load differs between patients with late-onset MD and controls. Increased lesion density in regions associated with cognitive and emotional functions may be crucial in late-onset MD, and vascular risk factors such as smoking may play an important role in the pathophysiology of late-onset MD, consistent with the vascular depression hypothesis.

Introduction

Several studies have demonstrated an increased frequency of cerebral white-matter lesions (WMLs) in patients with major depression (MD) compared with age-matched controls (Videbech, 1997), especially in late-onset and late-life MD (Herrmann *et al.* 2008). WMLs are identified as signal hyperintensities on proton-density or T2-weighted magnetic resonance imaging (MRI) and are traditionally classified based on their location and size (Fazekas *et al.* 2002). The lesions are thought to reflect underlying cerebrovascular disease (Pantoni & Garcia, 1997 ; Thomas *et al.* 2002) and positive correlations between WMLs, age

and vascular risk factors such as hypertension, diabetes and smoking have been demonstrated (Awad *et al.* 1986 ; Fazekas *et al.* 1993 ; Breteler *et al.* 1994 ; Liao *et al.* 1997). Women tend to have a higher WML load than men, and risk factors for WML development are seemingly gender specific (Sachdev *et al.* 2008), which is noteworthy regarding the higher prevalence of MD in women compared with men.

Although WMLs are implicated in a range of clinical conditions and also a frequent finding in healthy individuals past middle-age, their exact role in depression remains elusive because of differences in the reporting of lesion load and their relationship with confounding factors, such as age and vascular risk factors (Sachdev *et al.* 2008). In general, WMLs are associated with impaired cognitive functioning, for example in tasks of processing speed, memory and executive functions (Gunning-Dixon & Raz, 2000). In addition to cognitive impairment (Goodwin, 1997 ;

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Austin *et al.* 2001; Herrmann *et al.* 2007), subcortical WMLs in elderly depressed patients have been associated with a poor treatment outcome (Hickie *et al.* 1995; Steffens *et al.* 2001; Taylor *et al.* 2003*b*; Chen *et al.* 2006; Iosifescu *et al.* 2006) and a greater risk of subsequent dementia (Steffens *et al.* 2007).

The association between depression, WMLs and vascular risk factors is at the center of the vascular depression hypothesis (Krishnan & McDonald, 1995; Alexopoulos *et al.* 1997*a*; Krishnan *et al.* 1997) and in the later refined concept of subcortical ischemic depression (Krishnan *et al.* 2004). The vascular depression hypothesis states that WMLs contribute to the development of depression by disrupting the prefrontal systems or their modulating pathways, either by single, localized lesions or by an accumulation of lesions exceeding a certain threshold (Alexopoulos *et al.* 1997*a*), thus defining a subset of late-life depression closely related to vascular disease. However, the association between vascular risk factors and geriatric depression has been inconsistent (Kales *et al.* 2005), depending on study design. In particular, the choice of healthy control subjects is crucial, balancing the patient population distribution of vascular risk factors, as emphasized in a recent study by Sheline *et al.* (2008).

Over the past decade, research on WMLs and depression has shifted from focusing mainly on lesion load (i.e. lesion count, classification and volume) towards lesion localization. Recent research indicates that the exact localization of WMLs may be crucial in late-life MD (Sheline *et al.* 2008), and several studies of late-life MD have mainly found WMLs in subcortical regions and their frontal white-matter projections (Greenwald *et al.* 1998; MacFall *et al.* 2001; Taylor *et al.* 2003*a*; Videbech *et al.* 2004; O'Brien *et al.* 2006; Sheline *et al.* 2008), and in the basal ganglia (Videbech, 1997).

The aim of the present study was to investigate the association between the localization, number and volume of WMLs in a clinically well-described patient group with late-onset MD compared with a control group matched for age, gender and vascular risk factors. We tested the following hypotheses: (*a*) depressed patients have a greater lesion load (i.e. greater number and volume of WMLs) than controls, (*b*) depressed patients have more WMLs located in the frontostriatal region than controls, and (*c*) vascular risk factors have a significant effect on lesion load.

Method

Subjects

Patients were consecutively recruited from the Neuropsychiatric Clinic, Aarhus University Hospital, Risskov, Denmark and from out-patient psychiatric clinics in the County of Aarhus, Denmark. We examined 22 patients with late-onset, first-episode MD and 22 controls matched for age and gender and with no previous history of psychiatric illness. All patients met DSM-IV criteria (APA, 2000) for MD and ICD-10 criteria (WHO, 1993) for moderate to severe depression within 4 weeks of examination. Late-onset was predefined as the debut of depressive symptoms after the age of 50 years. Controls were recruited through advertisement and sought matched for age and gender with the patient group. All subjects were assessed with selected parts of the SCAN structured interview (Wing *et al.* 1998) at the inclusion and were rated for severity of depressive symptoms and psychomotor retardation with the Bech–Rafaelsen Melancholia Scale (MES; Bech, 2002) and the Widlöcher Depressive Retardation Scale (WDRS; Widlöcher, 1983) respectively; all controls were additionally screened with the Mini-Mental State Examination (MMSE; Folstein *et al.* 1975). 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 examination. All subjects were right-handed, except for three patients and one control who were ambidextrous. Records of prescribed medication, including psychotropics, were obtained. At the time of inclusion nine patients were treated with selective serotonin reuptake inhibitors (SSRIs), six with tricyclic antidepressants (TCAs), eight with serotonin-norepinephrine reuptake inhibitors (SNRIs) and seven with noradrenergic and specific serotonin antidepressants (NaSSAs). Eight out of 22 patients received more than one antidepressant, and one patient was treatment naïve at inclusion. In addition, nine patients received anxiolytics and/or sedatives such as benzodiazepines, three received antipsychotics and one received an antiepilepticum for mood stabilization. None of the controls received any psychotropic medication.

Exclusion criteria for both groups were organic brain disease (e.g. former stroke, cerebral vascular malformations, epilepsy, or other known neurological diseases), former brain injury, substance dependency and conventional contraindications to undergo MRI scanning. After a complete description of the study to the subjects, written informed consent was obtained. The study was approved by the regional ethical committee.

Vascular risk factors

Information on vascular risk factors was carefully obtained during the clinical interview and a composite

vascular risk score was calculated as defined in the Framingham Study (Wolf *et al.* 1991). Blood pressure (BP) was measured with an automated sphygmomanometer with a brachial cuff during rest and reported as a mean of three consecutive measures. Hypertension was defined as systolic BP $\geqslant$140 mmHg and/or diastolic BP $\geqslant$90 mmHg or the use of prescribed antihypertensive treatment. Diabetes was defined as fasting plasma glucose of minimum 7.0 mM and glycosylated hemoglobin (HbA1c) of minimum 6.2% or active antidiabetic treatment. Hypercholesterolemia was defined as lipid-lowering medication or fasting plasma values of total cholesterol >6.5 mM or low density lipoprotein (LDL) >4.0 mM or triglycerides >4.0 mM. Smoking status was divided into current, past (cessation at least 1 month prior to examination) or never. An estimate of lifetime tobacco consumption was measured in terms of so-called pack years (1 pack year being defined as a daily consumption of 20 cigarettes for 1 year). Tobacco other than cigarettes was converted to cigarette equivalents. Social class was determined according to education years and social position in Danish standards.

MRI and image analysis

MRI was performed on a whole-body 3-T Signa HDx GE scanner (GE Medical Systems, USA). The MRI protocol consisted of an axial fast spoiled gradient-echo (FSPGR) three-dimensional (3D) T1-weighted sequence [inversion time (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 for visualizing WMLs [echo time (TE)1 = 120 ms, repetition time (TR) = 8650 ms, TI = 2250 ms, FOV = 240 mm, matrix 224×256, slice thickness = 5 mm, gap = 1.5 mm] and an axial T2-weighted sequence (TE = 112 ms, TR = 5400 ms, FOV = 240 mm, matrix 416×416, slice thickness = 5 mm, gap = 1.5 mm).

The T1-weighted images were non-linearly transformed to match the Montreal Neurological Institute (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). The T2-weighted FLAIR images were rigidly matched to the T1-weighted volumes and then non-linearly transformed to standard space using the concatenation of the linear and non-linear transformations (Collins *et al.* 1994, 1995).

A prefrontal region of interest (ROI), defined as the cortical areas anterior to the precentral sulcus with their underlying white matter and cerebral structures, was manually labeled in the MNI standard brain and matched back to the original FLAIR images using the inverse subject-to-MNI space transformation. These manual labels were later used for WML volume and number estimation.

WMLs

Subcortical and deep WMLs, excluding periventricular WMLs, were identified on the FLAIR images by an experienced neuroradiologist (L.S.) blinded to subject status and were manually labeled on the FLAIR images using Display software (McConnell Brain Imaging Centre, MNI, McGill University, Canada). The individual lesion masks were resampled to the MNI standard space using the T1 transformation and blurred with a 4-mm full-width at half-maximum (FWHM) Gaussian kernel to model the lesion probability and the uncertainty of the manual labeling.

Statistical analyses

Differences between patients and controls in sociodemographic data and clinical variables were evaluated with the Wilcoxon two-sample rank sum test (Mann–Whitney test) for continuous variables and Fisher's exact test for categorical variables.

Lesion counts were analyzed with a negative binomial regression, as overdispersion invalidates the use of the usual Poisson regression. Lesion volume was modeled with a log-Gaussian regression model. The group variable was included first, as this was the factor of main interest, and sociodemographic and clinical variables such as age, smoking and hypertension were added when significant with a forward inclusion procedure. All analyses were carried out using Stata release 9.2 (Stata Statistical Software, StataCorp LP, USA) with two-sided tests and a 5% level of significance.

To analyze the localization of WMLs, a voxel-based analysis of the WML densities was performed using FMRISTAT (Worsley *et al.* 2002). 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 $t > 3.17$ ($p < 0.05$). All effects were tested on a voxel-by-voxel basis using two-tailed unpaired t tests, and t maps were examined for significant changes, including summary analyses of t-statistic images, based on the 3D Gaussian random field theory (Worsley *et al.* 1996). The effect of age and gender was studied using a voxel-wise regression analysis. The resultant images were reviewed in Register (McConnell Brain Imaging Centre).

Table 1. *Sociodemographic and clinical characteristics of patients with late-onset major depression and non-depressed controls*

	Patients (n=22)	Controls (n=22)	Statistics	
			z	p
Age (years), mean (S.D.)	57.4 (4.6)	59.2 (7.3)	0.5	0.64
Lifetime tobacco consumption (pack years), mean (S.D.)	21.4 (19.6)	6.6 (9.8)	−3.1	0.002[a]
Vascular risk factor score[b], mean (S.D.)	6.9 (4.7)	6.8 (4.4)	0.05	0.96
MES score, mean (S.D.)	16.5 (5.8)	0.3 (0.8)	−5.9	<0.001[a]
WDRS score, mean (S.D.)	16.0 (8.3)	0.5 (0.6)	−5.8	<0.001[a]
Gender, n (%)				1.00
Male	7 (31.8)	7 (31.8)		−
Female	15 (68.2)	15 (68.2)		−
Social class, n (%)				0.02[a]
High	12 (54.6)	20 (90.9)		−
Low	10 (45.5)	2 (9.1)		−
Hypertension, n (%)	11 (50.0)	12 (54.6)		1.00
Smoking, n (%)				0.02[a]
Current	11 (50.0)	3 (13.6)		0.02[a]
Past	8 (36.4)	9 (40.9)		1.00
Never	3 (13.6)	10 (45.5)		0.05[a]
Hypercholesterolemia, n (%)	8 (36.4)	9 (40.9)		1.00
Diabetes, n (%)	1 (4.6)	1 (4.6)		1.00
Cardiovascular disease, n (%)	3 (13.6)	0 (0.0)		0.23

MES, Bech–Rafaelsen Melancholia Scale (Bech, 2002); WDRS, Widlöcher Depressive Retardation Scale (Widlöcher, 1983); S.D., standard deviation.

[a] Significant results at $p < 0.05$.

[b] 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 hypertrophia.

The comparison of patients and controls, adjusted for age and gender, was initially performed for (*a*) only subjects with WMLs, and then for (*b*) all subjects, including subjects without visible WMLs. All analyses were performed in the whole-brain volume and in the prefrontal ROI alone.

Results

Sample characteristics

The sociodemographic and clinical characteristics of all subjects are presented in Table 1. Patients and controls showed an equal distribution of age and gender. Social class was, however, significantly different between groups ($p = 0.02$), with more patients belonging to lower social classes. There was a statistically significant difference in overall smoking status ($p = 0.02$) and lifetime tobacco consumption ($p = 0.002$) between patients and controls, the patient group featuring more current smokers and more pack years, and the control group featuring more never smokers. There was no significant difference in the remaining vascular risk factors between the two groups.

Lesion load

Analyses showed no significant difference in either the number ($p = 0.63$) or volume ($p = 0.65$) of WMLs between patients and controls. In addition, there was no significant difference in the prefrontal fraction of WMLs between groups, either regarding the number ($p = 0.18$) or volume ($p = 0.44$) of lesions. With a forward inclusion procedure, we found a significant positive effect of smoking history (i.e. current or past smoking) on the total number of WMLs ($p = 0.003$) and a significant positive effect of pack years on the total volume of WMLs ($p = 0.001$); smokers generally had a higher lesion load than subjects reporting that they had never smoked. Subsequent adjustment for smoking status or pack years did not change the conclusion of no significant difference in the number or volume of lesions between groups. Analyses were repeated for the prefrontal region alone with similar results (data not shown).

Fig. 1 displays the volumes of individual lesions in patients and controls; regression analysis showed no difference in the mean individual lesion size between the two groups ($p = 0.58$).
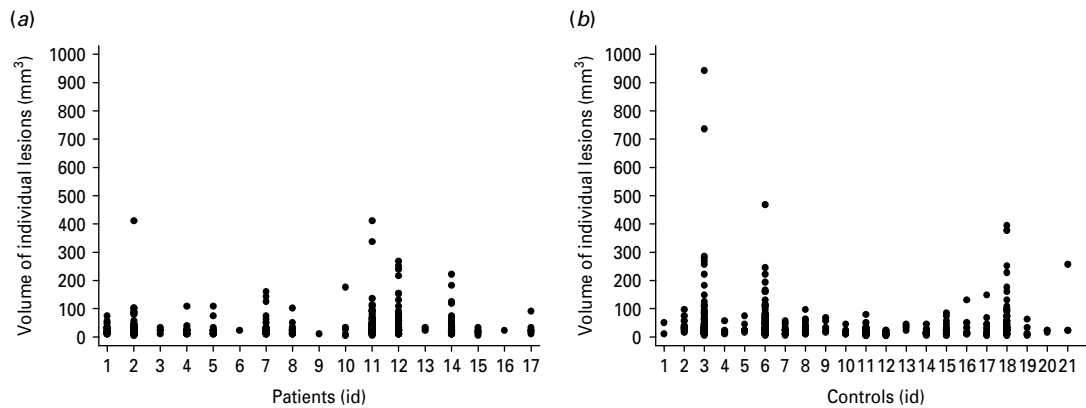
Fig. 1. Scatter plots of the volume of individual white-matter lesions (WMLs) in (*a*) depressed patients and (*b*) non-depressed controls. Only subjects with one or more WMLs are shown (17 patients and 21 controls, respectively). Statistical analysis showed no significant difference in individual lesion volume between groups.
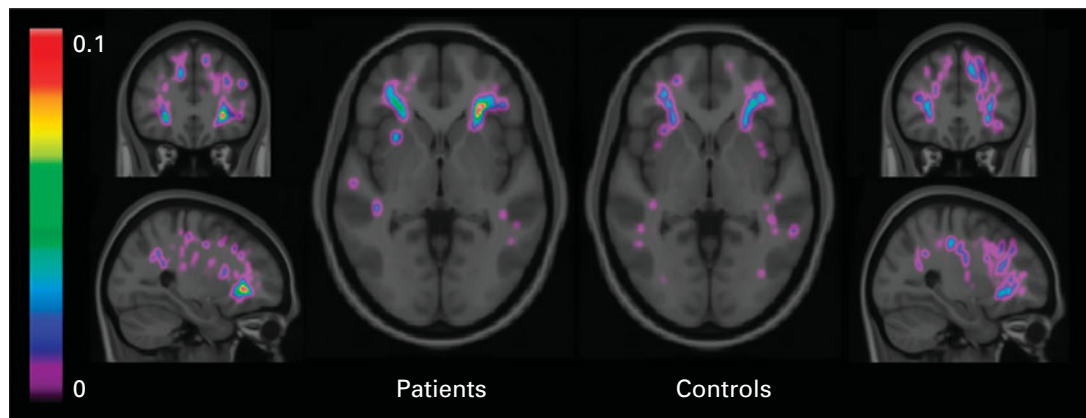

Fig. 2. Map in three planes (coronal, sagittal and axial views respectively) illustrating the average white-matter lesion (WML) density in patients (*left*) and controls (*right*) with one or more lesions (17 patients and 21 controls, respectively), adjusted for age and gender. The scale on the color bar is arbitrary as the average image is calculated from blurred lesion masks. In both groups, the highest lesion density is seen in the orbitofrontal white matter bilaterally.

Localization of lesions

Fig. 2 shows an average map of the lesion density in patients ($n=17$) and controls ($n=21$) with one or more WMLs, adjusted for age and gender, thus representing lesion localization. Subjects without lesions (five patients and one control) were excluded here, as our hypothesis on lesion localization was only relevant for subjects who presented with WMLs. The WMLs were widespread throughout the subcortical and deep white matter in both hemispheres, but most prominent in the orbitofrontal region bilaterally for both groups.

Voxel-based analyses on lesion density in subjects with one or more WMLs showed a significantly higher lesion density in the patients compared with the controls, adjusted for age and gender, in two regions: (1) the white-matter area just between the left precentral and postcentral gyrus ($p=0.031$) and (2) the right superior frontal gyrus ($p=0.037$), as illustrated in Fig. 3. A third region in the right inferior frontal gyrus with a tendency towards significance was also found ($p=0.091$). The subjects with WMLs in the two significant areas (clusters A and B in Fig. 3) were all current or past smokers. Clinical scores of depressive symptom severity (MES score) and psychomotor retardation (WDRS score) among the patients with one or more WMLs were not significantly correlated with the specific localization of WMLs in the two significant clusters, A (MES: $p=0.70$; WDRS: $p=0.66$) and B (MES: $p=0.28$; WDRS: $p=0.91$).

When including subjects without WMLs, voxel-based analyses showed no significant differences in the localization of WMLs between patients ($n=22$) and controls ($n=22$), adjusted for age and gender ($p=0.24$).
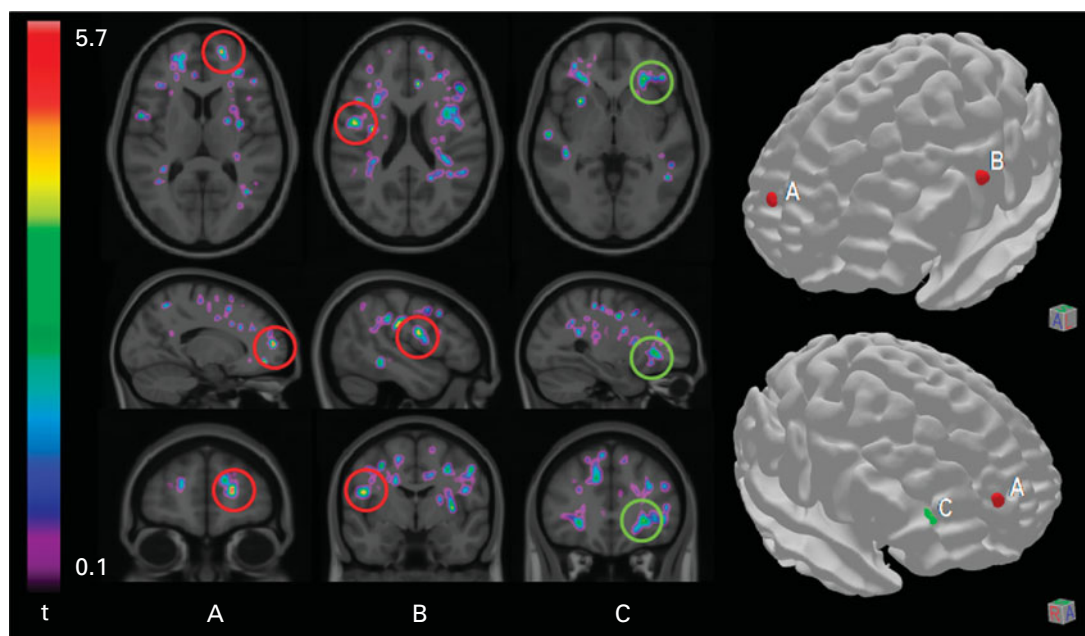
Fig. 3. Differences in lesion localization between depressed patients and non-depressed controls. The panel on the left shows selected slices of a *t*-statistic map representing the difference in mean white-matter lesion (WML) density in patients compared with controls among subjects with one or more lesions (17 patients and 21 controls, respectively), adjusted for age and gender. Setting the threshold at *t* > 3.17 yields two clusters, A and B (red circles), with significantly higher WML density in patients compared with controls (*p* < 0.05, corrected). A third cluster, C (green circles), with a tendency towards significance (*p* = 0.091) is also shown. The clusters are illustrated in the right panel, where the semi-transparent display allows the clusters to be seen more three dimensionally in oblique left (anterior-left) and oblique right view (anterior-right). The images are created by overlaying a non-transparent rendering of clusters on a semi-transparent surface rendering of a standard brain. The significant red clusters are localized in the right superior frontal gyrus corresponding to the most frontal projections of the corpus callosum (A) and near the left precentral sulcus, on the rim to the left parietal operculum, corresponding to the course of the left superior longitudinal fasciculus (B). The yellow cluster (C) with a tendency towards significance is situated in the right inferior frontal gyrus corresponding to the course of the right inferior fronto-occipital fasciculus and the right uncinate fasciculus.

Testing the effect of age and gender on lesion density revealed a significant interaction between age and gender on lesion density (*p* < 0.05); that is, the pattern of age-related lesion localization differed between women and men in both groups.

Discussion

In this MRI study on cerebral WMLs, patients with late-onset, first-episode MD were carefully matched with a non-psychiatric control group in terms of age, gender and several vascular risk factors. We detected extensive white-matter changes across all subjects, but found no significant difference in the number and volume of lesions between the two groups, contradicting our initial hypothesis. Although our findings show a high density of frontal WMLs across all subjects, we found a significant difference in the localization of the WMLs between patients and controls. Given the equal lesion load, our findings therefore indicate that, in terms of depression and WMLs, lesion localization is more important than lesion count

or volume, as predicted in the second hypothesis. Of note, five patients and one control had no visible WMLs, suggesting that WMLs are not a necessary condition for late-onset MD but may contribute to the pathogenesis in a subgroup of late-life MD, in accordance with the vascular depression hypothesis (Krishnan & McDonald, 1995; Alexopoulos *et al.* 1997*a*; Krishnan *et al.* 1997).

Among the subjects with one or more WMLs, the patients displayed a significantly higher lesion density than the controls in two areas, A (*p* = 0.037) and B (*p* = 0.031), as illustrated in Fig. 3. A third cluster, C, showed a tendency towards significance (*p* = 0.091). Cluster A is localized in the right superior frontal gyrus, corresponding to the most frontal projections of the corpus callosum. This is a boundary zone of white matter underlying the medial prefrontal cortex (MPC), the dorsolateral prefrontal cortex (DLPFC), the anterior cingulate cortex (ACC) and the orbitofrontal cortex (OFC). The adjacent frontopolar region approximates to Brodmann area (BA) 10 (Brodmann & Gary, 2006). These prefrontal areas and their

subcortical circuits are thought to play an important role in various aspects of cognition, for example in decision making (Krawczyk, 2002), in the regulation of emotional behavior (Phillips *et al.* 2008), and in the strategic processes involved in memory retrieval and executive functions such as planning, abstract thinking and initiating appropriate actions (Salloway *et al.* 2001). Cognitive deficits relating to all areas have been described in the depression literature (Videbech *et al.* 2002; Rogers *et al.* 2004; Drevets, 2007).

Cluster B is localized in the white-matter area between the left precentral and postcentral gyrus, corresponding to the course of the left superior longitudinal fasciculus, on the rim to the left parietal operculum. This region approximates to BA6, which comprises the premotor cortex and supplementary motor area (SMA), and is traditionally thought to be involved in the planning of complex, coordinated movements in interaction with the primary motor cortex (Chouinard & Paus, 2006). However, more recent research indicates that cortical motor circuits also contribute to different aspects of voluntary action, that is as a form of decision making, through complex networks involving the prefrontal cortex, the motor and sensory cortices, in addition to the parietal cortex (Haggard, 2008). As lack of volition is a key feature of depression, it is tempting to hypothesize that impairment of motor circuits may play a role in MD. In addition, the involvement of motor circuits might explain the frequent symptom of psychomotor retardation seen in late-onset MD (Alexopoulos *et al.* 1997*b*); we could, however, not demonstrate any association between psychomotor retardation, measured by WDRS, and the presence of WMLs in cluster B.

Cluster C is situated in the right inferior frontal gyrus, corresponding to the course of the inferior fronto-occipital fasciculus and the uncinate fasciculus, the latter linking the frontal lobe to the anterior portion of the temporal lobe. Although not significant in our analysis, the tendency towards significance warrants a comment. The area approximates to the lateral OFC (BA 47), which plays a major role in the human reward system (Rolls, 2000; Krawczyk, 2002), and dysfunction of this reward system has been hypothesized to prompt the anhedonic symptoms of depression (Naranjo *et al.* 2001). Overall, our findings indicate that WMLs in late-onset MD may affect specific cerebral areas involved in both complex cognitive tasks and voluntary action.

Our study differs from previous studies of WMLs in several aspects. First, periventricular lesions (PVLs) were omitted from the assessment, and thus our analyses only included subcortical and deep WMLs. We made this decision on the basis of existing literature indicating that neuropathological differences exist between deep WMLs and PVLs (Fazekas *et al.* 1993), and that deep WMLs rather than PVLs are most strongly associated with depressive symptoms, consistent with the vascular depression hypothesis (Krishnan *et al.* 2006). Deep WMLs have also been found to be more common in elderly subjects presenting with late-onset MD (O'Brien *et al.* 1996). Similarly, in neuropathological analyses of brains from people with MD, subcortical WMLs were all ischemic (Thomas *et al.* 2002) whereas PVLs had multiple causes (Thomas *et al.* 2003).

The fact that we found no difference in the number or volume of WMLs between patients and controls is consistent with findings by Videbech *et al.* (2004), but contrary to numerous previous studies reporting an increased burden of WMLs in depression (Videbech, 1997; Herrmann *et al.* 2008). This might be because our study was underpowered for this specific question or because our patients and controls were matched for several vascular risk factors, which is contrary to the design of many previous studies. Furthermore, the high prevalence of WMLs in our control group is supported by larger population studies such as the Rotterdam Scan Study and the Atherosclerosis Risk in Communities Study (ARIC), which report a high prevalence of WMLs in a general study population (Liao *et al.* 1997; de Leeuw *et al.* 2001). Unexpectedly, our analyses showed a significant interaction between age and gender on lesion density, which we accounted for, and the gender-dependent effect of age on lesion localization must therefore be considered in future studies of late-onset and late-life MD.

Despite a significant difference in smoking status between groups, with almost 3.7 times more current smokers in the patient group, the patients and controls in our study have very similar composite vascular risk scores as defined by the Framingham Study (Wolf *et al.* 1991). This illustrates the importance of evaluating each vascular risk factor independently, because an otherwise clinically well-established composite score introduces the risk of masking the effect of a single exposure variable such as smoking. Accordingly, in the present study, smoking and lifetime tobacco consumption, measured as pack years, had the most significant effect on the number and volume of WMLs respectively, consistent with our third hypothesis. The co-morbidity between smoking and depression is well known, but complex, and is influenced by socio-demographic differences, such as lower socio-economic status (Swan & Lessov-Schlaggar, 2007). It has been suggested that the relationship between smoking and MD is mediated largely by genetic factors, predisposing to both smoking and MD (Kendler *et al.* 1993). Smoking is also considered a risk factor for WMLs, possibly on the basis of long-term damaging

effects attributed to atherosclerosis and oxidative stress (Jane-Llopis & Matytsina, 2006). Of interest, in a community study of 228 men and 208 women aged 60–64 years, Sachdev *et al.* (2007) found that WMLs were more common in women with current smoking as a significant determinant, whereas hypertension, heart disease and high homocysteine levels were significant determinants in men. Given the higher prevalence of MD in women, this raises an important question about the role of smoking in the pathophysiology of depression, and whether late onset of depressive symptoms may be related to smoking habits and cumulative tobacco load. Although speculative, a high prevalence of smoking in the patient group may also indicate a smoking-related vulnerability towards depressive symptoms through yet undefined mechanisms, perhaps by predisposing to localized damaging effects on the brain microstructure, presenting as WMLs in brain regions particularly vulnerable in MD. Unfortunately, the cross-sectional design of our study does not allow us to conclude further on the confounding effects of smoking in late-onset MD.

Our study has several strengths. First, the equally high lesion count in patients and controls adds power to the significant findings in lesion localization. Second, our patients are consecutive, diminishing selection bias. The gender distribution resembles that of depression in the general population, that is two-thirds are women. We use a personal structured interview, in contrast to self-rating scales, to confirm diagnosis and other medical data according to international diagnostic criteria. In addition, we confine the inclusion criteria to first-episode and late-onset disease to ensure a more homogeneous patient group. All subjects are well characterized based on clinical examination, MRI scanning and laboratory test results. Finally, the distribution of vascular risk factors is very similar in the two groups, except for smoking status, which is very important in terms of possible confounding effects as emphasized above.

However, our study also contains some limitations. We used a manual MRI segmentation method for the WMLs, which is time-consuming and prone to inter- and intra-rater variability; by using a single rater, the former was eliminated in this study. Although several automated segmentation methods exist, many still have limited accuracy for different lesion types (i.e. subcortical, deep and periventricular lesions). As we chose to ignore the PVLs for reasons described above, we preferred the manually labeled lesions for analysis in this study. The T2-weighted FLAIR sequence used for segmentation of WMLs had a 1.5-mm gap between slices and a slice thickness of 5 mm. These parameters, although consistent through the material, are not ideal and may cause sampling errors with respect to the definition of the WMLs. We defined late onset by a lower age limit of 50 years, which is younger than the majority of other studies, which have a lower age limit of 60–65 years. Significantly more patients than controls were classified as belonging to a lower social class, which supports epidemiological reports of an inverse correlation between socio-economic factors and mental illness, including depression (Hudson, 2005); however, this causality is complex, as lower socio-economic status can be both a predictor and a consequence of depression. Our patients, except for one, were not treatment naïve regarding psychotropic drugs, but this is not considered important for the development of WMLs (Videbech, 1997; Herrmann *et al.* 2008). Finally, the size of our sample may limit the statistical power of our findings, increasing the risk of type 2 statistical errors. We cannot preclude that the lack of significant between-group differences on lesion load may be due to an insufficient sample size.

In conclusion, we found that more patients with first-episode late-onset MD than non-depressed controls have WMLs localized in the frontostriatal area, despite an equal lesion load and similar vascular risk score. Our results suggest that the lesion localization is more important than the lesion load, and that the effect of age on lesion localization is gender dependent. We also show that vascular risk factors such as smoking have an effect on the number and volume of WMLs, and thus our study supports the vascular depression hypothesis in terms of an association between WMLs and depression, with smoking (past or present) as an important risk factor. A greater emphasis on vascular risk factors and their impact on cerebral microstructure may contribute to a more differentiated diagnostic approach to MD, with implications for future prevention and treatment.

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Declaration of Interest

None.

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