The spatial distribution of age-related white matter changes as a function of vascular risk factors—Results from the LADIS study

E. Rostrup a,b,c,⁎, A.A. Gouw d, H. Vrenken d, E.C.W. van Straaten d, S. Ropele e, L. Pantoni f, D. Inzitari f, F. Barkhof d, G. Waldemar g and on behalf of the LADIS study group

a Danish Research Centre for Magnetic Resonance, Copenhagen University Hospital, Hvidovre, Denmark
b Functional imaging unit, Department of Clinical Physiology and Nuclear Medicine, Copenhagen University Hospital, Glostrup, Denmark
c Center for Healthy Aging, University of Copenhagen, Denmark
d Department of Radiology and Image Analysis Centre, VU Medical Centre, Amsterdam, The Netherlands
e Department of Neurology, Medical University, Graz, Austria
f Department of Neurological and Psychiatric Sciences, University of Florence, Florence, Italy
g Memory Disorders Research Group, Department of Neurology, Copenhagen University Hospital, Copenhagen, Denmark

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ABSTRACT

White matter hyperintensities (WMH) are a frequent finding on brain MRI of elderly subjects, and have been associated with various risk factors, as well as with development of cognitive and functional impairment. While an overall association between WMH load and risk factors is well described, possible spatially restricted vulnerability remains to be established.

The aim of this study was to investigate the spatial distribution of WMH in normally functioning elderly subjects. We introduce a voxel-based approach in which lesion probability is mapped as a function of clinical risk factors using logistic regression, and validate the method using simulated datasets. The method was then applied in a total of 605 participants of the LADIS study (age 74±5 years, all with WMH), and the location of manually delineated WMH was investigated after spatial normalisation. Particularly strong and widespread associations were found for age, gender and hypertension. Different distribution patterns were found for men and women. Further, increased probability was found in association with self-reported alcohol and tobacco consumption, as well as in those with a history of migraine. It is concluded that the location of WMH is dependent on the risk factors involved pointing towards a regionally different pathogenesis and/or vulnerability of the white matter.

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Introduction

White matter hyperintensities (WMH) are frequently seen on brain MRI of elderly subjects (de Leeuw et al., 2001). Although these lesions may be found in healthy subjects, their presence and severity has been associated with cognitive disorders, gait and balance disorders and depression (de Groot et al., 2000) (Guttmann et al., 2000; Whitman et al., 2001). Associations with measurements of atherosclerosis, i.e. intima–media thickness of the carotid arteries (Manolio et al., 1999), retinopathy (Wong et al., 2002) and changed endothelial marker profiles (Hickie et al., 2005; Obiadege and Saver, 2006) have further strengthened this concept.

The most important risk factors for WMH are increasing age (Breteler et al., 1994; Liao et al., 1997; Schmidt et al., 2000) and hypertension (Basile et al., 2006; De Leeuw, 2004; Jeerakathil et al., 2004; Longstreth et al., 1996). In the majority of studies, males are reported to have more WMH than females, but in general gender does not appear to be a strong risk factor (Longstreth et al., 1996). Evidence of prior (cerebro)vascular or heart disease, increased homocysteine levels (Hassan et al., 2004; Vermeer et al., 2002) and lower forced expiratory volume (Longstreth et al., 1996) have also been reported as risk factors of WMH. Other risk factors, such as diabetes mellitus, hyperlipidemia, smoking, high body mass index, decreased vitamin B12, and alcohol have yielded inconsistent associations with WMH (Breteler et al., 1994; Hickie et al., 2005; Jeerakathil et al., 2004; Liao et al., 1997; Longstreth et al., 1996, 2005; Murray et al., 2005; Stenset et al., 2006).

Recent studies have emphasised the differences in risk factor profile between large and small vessel disease, and between different subtypes of small vessel disease (Jimenez-Conde et al., 2010; Khan et al., 2007).
These studies indicate that while age and hypertension are strongly associated to the occurrence of WMH, factors such as hyperlipidemia and diabetes may relate less strongly or even inversely to WMH.

WMH arise in different locations through the brain. Traditionally, a distinction has been made between periventricular and subcortical WMH. It has been suggested that subcortical WMH could be more associated with hypertension and other vascular risk factors, while periventricular WMH have been considered a phenomenon related to more general age processes, including more benign structural changes in sub-ependymal white matter (Scheltens et al., 1993). However, later studies have found no differential association with vascular risk factors, and have suggested that the division between periventricular and subcortical regions/WMH is arbitrary (Barkhof and Scheltens, 2006; DeCarli et al., 2005). Therefore, the impact of WMH location is often assessed by dividing the brain into different brain regions, i.e. the frontal, parietal, occipital and temporal lobes, basal ganglia and infratentorial region.

The concept of a spatially varying vulnerability to WMH is important because it might reflect differences in pathophysiological mechanisms, and as such be of potential use to the design of clinical intervention. Furthermore it may help inform the interpretation of clinical MR scans showing WMH, and to establish a firmer basis for defining patterns related to normal versus pathological ageing.

Few studies however, have investigated these relationships between risk factors and WMH localisation. The WMH distribution in the presence of hypertension has been studied, but is still unclear. In one study, hypertensive subjects had a higher load of periventricular WMH and frontal-temporal WMH in comparison to normotensive subjects (Wiseman et al., 2004). On the contrary, other investigators showed that hypertension is correlated with subcortical WMH (Murray et al., 2005) or found no difference in WMH pattern at all (Enzinger et al., 2006); One previous study considered the distribution of WMH using a truly voxel-based approach (Enzinger et al., 2006). However, this study focused on the differential distribution of WMH, when classified according to a visual rating scale, and considered only a limited set of vascular risk factors. To our knowledge, other risk factors than age and hypertension have not been studied in detail with respect to WMH location. Voxel-wise statistical analysis was used in another previous study (Holland et al., 2008) to compare the distribution of WMH in patients with dementia or amyloid angiopathy vs. controls. Furthermore, one study considered the impact of low cardiac output on the distribution of WMH, but did not analyse the distribution by voxel-wise statistical scores (Jefferson et al., 2011).

Therefore the aim of this study is to characterise the spatial distribution of lesion probability as a function of individual or groups of risk factors using a voxel-wise analysis. While this approach avoids the bias associated with predefined regions of interest, it is also complicated by the binary distribution of the outcome variable (i.e., either absence or presence of a lesion), which precludes the use of standard linear regression methods. The few studies that have attempted lesion–probability mapping (LPM) using a linear approach, therefore have combined this with permutation-based inference (Enzinger et al., 2006). In the present study we present another approach, using direct fitting of a logistic function describing the lesion probability in each voxel. In this study we present data from a large cohort of normally functioning elderly subjects with white matter abnormalities, and use the logistic regression method to characterise the influence of risk factors on the specific risk of having a lesion at a given location.

Methods

Study population

Data were drawn from the multi-centre, multinational Leukoaraisis and Disability (LADIS) study. The LADIS project studies the role of WMH as an independent predictor of the transition to disability in initially non-disabled elderly. The rationale and design of the LADIS study have been described elsewhere (Pantoni et al., 2005). In short, 639 elderly subjects who had no or only mild disability in their instrumental activities of daily living (IADL) were enrolled. Subjects presented with complaints including mild memory loss, minor motor disturbances, minor focal cerebrovascular events, or non-specific reasons for undergoing a cranial neuroimaging study (with WMH as an incidental finding).

Subjects were selected and stratified for grade of WMH severity—according to the categorization into three severity classes of the modified Fazekas scale (Fazekas et al., 1987)—to balance subject groups with mild, moderate and severe WMH. All subjects were assessed using an extensive set of clinical and functional tests including global functioning, cognitive, motor, psychiatric, and quality of life measures.

Vascular risk factors

To assess vascular risk factors, a structured and comprehensive data questionnaire was used together with a review of available records by trained medical personnel (Basile et al., 2006). Both categorical and continuous variables were used as risk factors in the present paper. The continuous variables were age, years of education, blood pressure (systolic, BPSYS and diastolic, BPDIAG), body mass index (BMI), cumulative smoking (self-reported number of packs pr. year times years of smoking) and cumulated alcohol intake (self-reported daily alcohol intake (g/day) multiplied by years of drinking). Additionally, the haemoglobin concentration and total cholesterol concentration were used. Blood pressures as well as biochemical parameters were measured prior to or on the day of the clinical interview.

The categorical variables included: gender, history of hypertension (subjects receiving antihypertensive treatment or with values ≥140/90 mm Hg, based on measurements taken on several separate occasions), and presence of diabetes mellitus (treatment with antidiabetic medications, or at least 8-hour fasting plasma glucose ≥ 7.0 mmol/L or 126 mg/dL). Further diagnostic categorisations included presence of chronic obstructive pulmonary disease (COPD) or of migraine with or without aura. Migraine was diagnosed on the basis of standard criteria (Cephalalgia, 1988). A history of previous stroke was not included in the models, as it was considered a result of WMH risk factors, rather than a separate risk factor.

As in any multicentre study it was not possible a priori to determine whether any centre effects were to be interpreted as true population differences (i.e., a separate risk factor e.g. with a genetic basis) or as a result of different sampling at different centres leading to different observed risk factor profiles in different centres (i.e. colinearity exists between centre and risk factor variable). In the analysis of total lesion load, we therefore considered models with and without inclusion of the centre effect, and interpreted unchanged covariate effects as a particularly strong evidence of a centre independent effect.

Finally, we considered the effect of the total WMH burden (lesion load), estimated as the logarithm of the volume of WMH. While not a vascular risk factor per se, this variable reflects the combined effects of other risk factors, and correction for it may serve to emphasise any spatial specificity in lesion susceptibility (Vellinga et al., 2009) (Holland et al., 2008). We therefore consider both results with and without this correction.

MRI examinations

MRI examinations were performed at 1.5 T in ten centres and at 0.5 T in one centre. Each MRI examination included fluid attenuated inversion recovery (FLAIR) images (TE 100–140 ms, TR 6000–10,000 ms, TI 2000–2400 ms, voxel size 1 × 1 × 5–7.5 mm3, interslice gap 0.5 mm, 19–24 slices, FoV 250 mm, matrix size 256 × 256), on which all WMH
measurements were performed, and axial T2-weighted images (TE 100–120 ms, TR 4000–6000 ms, voxel size 1 × 1 × 5–7.5 mm³, 19–24 slices, FOV 250 mm, matrix size 256 × 256) that were used for reference. All scans were transferred to the Image Analysis Center (IAC) in Amsterdam, the Netherlands, for central data storage and region-of-interest (ROI) analysis (WMH volumetry), while further post-processing and analyses were performed in Copenhagen, Denmark.

Volumetric analysis of WMH was performed by a trained rater (ECWvS) (van Straaten et al., 2006). Using home-developed software (Show_Images, version 3.6.1) on each slice of the FLAIR images, the lesions were marked with a “seed” and outlined using local thresholding. When necessary, borders could be adjusted by the operator by changing thresholds for upper and lower intensity values. This procedure was carried out for each slice individually, but the data was eventually stored as 3D binary files, which will be referred to as ROI-data. Intra-observer reliability was assessed by measuring WMH volumes twice on 18 scans with equal distribution across the Fazekas categories. The intra-class correlation coefficient was 0.99.

High resolution T1-weighted structural scans were obtained in some subjects (images (TE = 4–7 ms; TR = 10–25 ms; TI = 100–950 ms; flip angle = 10–30°; voxel size = 1 × 1 × 1.5 mm³; FOV = 250 mm²)). These data were analysed using FSL sienax software (www.fmrib.ox.ac.uk/fsl/siena), producing a volume scaling factor which is inversely related to absolute intra-cranial volume, as well as the brain volume after normalisation to standard intracranial volume (relative brain volume).

**Lesion probability maps**

The image data were spatially normalised using a stepwise procedure in which the linear transformation that co-registered each individual’s FLAIR image to the corresponding T2-weighted image was first determined using SPM2 routines. Subsequently, each individual T2-weighted image was spatially normalised, using non-linear transformations, to the standard T2-weighted template of SPM2 (www.fil.ion.ucl.ac.uk/spm/spm2). In a second pass the average of the normalised T2-weighted images served as a study specific template to which the T2-weighted data was spatially normalised. The corresponding FLAIR images and ROI-data were normalised to the same space using the appropriate combination of transformation matrices, creating isotropic voxels of 2 × 2 × 2 mm, and using nearest neighbour interpolation for the ROI-data to preserve the binary nature of the data. The second pass was performed using non-linear alignment tool of the FSL package (www.fmrib.ox.ac.uk/fsl/fniirt) (Andersson et al., 2007).

Maps of the average distribution of WMH for the entire group, as well as for subgroups, could be calculated by simple summation of the ROI data. However, in order to perform parametric comparisons, as well as to calculate the predicted distribution at a given level of explanatory variables, the WMH distribution was described using a multiple logistic regression model (Glantz and Slinker, 2000). For each voxel k in template space, the model expresses the probability of that location being classified as hyperintensity as

\[ P_k = \frac{1}{1 + e^{-X_k \beta}} \]  

where \( X_k \) is a row vector of independent variables for subject s, and \( \beta \) is the parameters to be determined for voxel k. The vector \( X_k \) has length \( N_{\text{cov}} \), i.e. the number of clinical parameters included in the model plus an offset. The parameter vector \( \beta \) is a column vector of length \( N_{\text{cov}} \). Eq. (1) is equivalent to expressing the logarithm of odds for the occurrence of WMH as a linear combination of the independent variables \( \log(\text{odds}) = X_k \beta \). In practice the parameters are determined by a maximum likelihood estimate, where the logarithm of likelihood of the observed data set is expressed as:

\[ \log(L) = \sum_{s=1}^{N_{\text{subj}}} (X \beta) Y_{s,k} - \sum_{s=1}^{N_{\text{subj}}} \log \left( 1 + e^{-X \beta} \right) \]  

where \( N_{\text{subj}} \) is the number of subjects in the cohort, and \( Y_{s,k} \) is a binary-valued vector of length \( N_{\text{subj}} \), describing the observed occurrence of WMH in location \( k \).

The significance of individual regressors or groups of regressors (like those arising from categorical variables), can be tested by calculating

\[ Q = 2 \cdot (\log(L) - \log(L_0)) \]  

where \( L_0 \) is the likelihood of the observations given a reduced model that excludes the regressors of interest. The associated p-value can be estimated by assuming Q follows an approximate \( \chi^2 \) distribution. Furthermore, the goodness-of-fit was estimated using the Hosmer–Lemeshow statistic, for which high values, according to the corresponding \( \chi^2 \)-distribution, indicate a poor fit. Apart from the p-value criterion, significance was accepted only where the goodness-of-fit variable was non-significant, and for locations where WMH occurred in at least 5 subjects.

**Statistical analyses**

The independence of overall lesion load on various risk factors was investigated using a multiple linear regression model. Regression was performed using StataSE v 11.1. The data was analysed either by pooling data from all centres, or by estimating the within centre effect (correcting each parameter by the mean value within centre). The within centre analysis was performed both with lesion load data obtained in native and in normalised space. The latter measurement of lesion load is expected to be independent on brain volume.

Voxel-based analysis assessed the spatial distribution of lesion risk, and was performed using two levels of model complexity. The simplest model encompassed univariate testing of each covariate. In order to investigate the specific effect of each covariate an additional analysis was performed, in which the covariate of interest as well as the overall lesion load (logarithm of lesion volume, as obtained in normalised space) was included. Multivariate analysis was avoided in general due to the complicated linearity structure of the various risk factors, as well as a rapidly increasing computation time for large design matrices, when using the permutation based approach to statistical inference. However, one multivariate analysis was included for comparison, based on conventional thresholding.

Each model included an offset parameter representing the hypothetical lesion probability at zero value of all other parameters.

Variables that showed a strongly right-skewed distribution (such as total WMH volume, alcohol consumption and smoking) were transformed logarithmically prior to the analysis. Furthermore, all continuous variables were normalised to a standard deviation of 1; this enabled direct comparison of effect sizes between variables measured in different units.

The resulting maps of association strength, Q, were evaluated for statistical significance using the “threshold free cluster enhancement” technique (TFCE) (Smith and Nichols, 2009). In short, this methodology combines cluster volume and amplitude information into a single parameter, the TFCE-score, by integrating over a range of thresholds for the statistical map. The distribution of TFCE-scores under the null-hypothesis was investigated using random permutation analysis, with 1500–2000 iterations. The TFCE-maps were thresholded at \( p < 0.01 \). Due to the complexity of the calculations it was not possible to run the permutation analysis for each design matrix separately. Instead the distribution was determined for five continuous (age, diastolic and
systolic blood pressure, cholesterol, smoking) and three binary variables (gender, migraine and hypertension), and was determined to depend mainly on the degrees of freedom. Thus, these results were used for thresholding other continuous and binary variables.

Results

A total of 605 subjects could be included in the analysis, while 34 had to be excluded due to missing FLAIR or T2-weighted scan, inadequate volume coverage or image contrast. The subjects included had a median age of 73.8 years (range of 64.1–85.4), and a gender distribution of 282 men/323 women. The 11 recruiting centres were represented evenly in the data set, contributing from 8 to 11% of the subjects each. The MRI was performed an average of 11.4±2.8 (median 7) days after inclusion and the clinical interview.

Further demographic data, including mean values of the covariates used, are given in Table 1. The full dataset was not available for all subjects, e.g. measurements of cholesterol were only available in 519 subjects. The data was missing at random due to variable clinical procedures and logistic issues, rather than to differential treatment of specific groups of subjects or in specific centres.

Lesion load

Multivariate analysis of lesion load (Table 2) showed a pattern of strongly significant associations with age and hypertension, and less pronounced associations with gender (males higher lesion load) and smoking. Diastolic blood pressure had a significant effect in the within centre analysis. Effects of alcohol intake and haemoglobin concentration were only significant when pooled across centres, and may thus have been influenced by centre effects. No significant associations were found for the presence of either pulmonary disease (chronic obstructive pulmonary disease, COPD), diabetes or hyperlipidemia (whether based on measured blood values or diagnostic categories).

Brain volume and head size measurements were available only for subjects for whom a high quality 3D structural scan had been obtained. In this subgroup (n=335, see lower rows of Table 2) there was a presumably trivial relation between larger brain size and higher lesion load, likely to be related to the larger volume of white matter. However, even when lesion load was calculated after normalisation to standard space, a weak significant association with head size remained.

WMH distribution

The average distribution of WMH is shown in Fig. 1. Very frequent occurrence of WMH was noted in the area around the anterior horns of the lateral ventricles, from where it stretched posteriorly along their lateral borders. High lesion probabilities were also noted in a deep white matter region more lateral, extending towards the precentral gyrus. As expected, WMH were also frequent around the posterior horns of the ventricles, as well as in the centrum semiovale.

Univariate analyses

The distribution of voxels with significant correlations between WMH frequency and each covariate is shown in Fig. 2 (panels A–L). Volumes of significant areas as well association strengths are shown in Table 3. As expected, a particularly strong association was found with the age of the subject (Fig. 2, panel A), where the location of significant voxels corresponded roughly to the areas of frequent WMH. Within the significant areas, the median increase in odds ratio was 36% at one standard deviation above the sample mean (Table 1), or about 6% per year of age.

Gender was found to display a slightly more complex pattern (panel B) in that men had a higher risk of lesion especially around the anterior and posterior horns of the lateral ventricles, but that by contrast, in deep white matter below the central sulcus, the risk was lower for men than women. The variable ‘years of education’ was also a significant risk factor, showing lower risk associated with more years of education, predominantly in white matter underlying frontal regions (panel C).

The variables related to blood pressure (panels D–F) showed scattered areas of significant association with WMH in deep white matter as well as in periventricular white matter. There was a stronger association with diastolic than with systolic blood pressure, but both variables had small effect sizes. However, there was a more widespread association with the variable hypertension than with the measured blood pressures; when uncorrected for other variables the median odds ratio in the significant region was 64% for subjects fulfilling the criterion of hypertension (Table 3).

### Table 1

Demographic data for the subjects included in the study.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Type</th>
<th>Values</th>
<th>Availability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Mean ± std.dev.</td>
<td>74.1±5.1</td>
<td>605</td>
</tr>
<tr>
<td>Education (years)</td>
<td></td>
<td>9.6±3.8</td>
<td>604</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td></td>
<td>149.1±20.4</td>
<td>605</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td></td>
<td>83.6±11.1</td>
<td>605</td>
</tr>
<tr>
<td>Lacunes (number)</td>
<td></td>
<td>1.5±2.9</td>
<td>605</td>
</tr>
<tr>
<td>Haemoglobin concentration (g/dL)</td>
<td></td>
<td>14.1±1.3</td>
<td>528</td>
</tr>
<tr>
<td>Cholesterol concentration (g/dL)</td>
<td></td>
<td>5.6±1.1</td>
<td>519</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>Median, interquartile range</td>
<td>51.1 – 477</td>
<td>601</td>
</tr>
<tr>
<td>Alcohol intake, cumulated smoking, pack years</td>
<td></td>
<td>0 – 768</td>
<td>605</td>
</tr>
<tr>
<td>WMH, Fazekas grade</td>
<td>Grade 1/2/3</td>
<td>268/186/151</td>
<td>605</td>
</tr>
<tr>
<td>Gender, Male</td>
<td>Number (%)</td>
<td>282 (46.6%)</td>
<td>605</td>
</tr>
<tr>
<td>Stroke (one or more)</td>
<td></td>
<td>186 (29.7%)</td>
<td>605</td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td>439 (70.1%)</td>
<td>598</td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td>90 (14.4%)</td>
<td>596</td>
</tr>
<tr>
<td>Migraine w/o aura</td>
<td></td>
<td>59 (9.8%)</td>
<td>605</td>
</tr>
<tr>
<td>Migraine with aura</td>
<td></td>
<td>39 (6.5%)</td>
<td>605</td>
</tr>
<tr>
<td>COPD</td>
<td></td>
<td>60 (9.9%)</td>
<td>605</td>
</tr>
</tbody>
</table>

Availability is the number of subjects for which both an appropriate image dataset, as well as a record of the given variable was available.

### Table 2

Multivariate analysis of lesion load vs. risk factors.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Lesion load, native space (n = 544)</th>
<th>Lesion load, normalised (n = 528)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Across centres</td>
<td>Within centres</td>
</tr>
<tr>
<td>Age</td>
<td>0.018***</td>
<td>0.016***</td>
</tr>
<tr>
<td>Male gender</td>
<td>0.113*</td>
<td>0.109*</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.143***</td>
<td>0.119**</td>
</tr>
<tr>
<td>Bp, diastolic</td>
<td>0.002</td>
<td>0.003*</td>
</tr>
<tr>
<td>log(Alcohol)</td>
<td>0.054*</td>
<td>0.003</td>
</tr>
<tr>
<td>log(Buttab)</td>
<td>0.030*</td>
<td>0.033**</td>
</tr>
<tr>
<td>Education</td>
<td>−0.007</td>
<td>−0.031</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>−0.043*</td>
<td>−0.022</td>
</tr>
<tr>
<td>Migraine w/o aura</td>
<td>0.054</td>
<td>0.019</td>
</tr>
<tr>
<td>Rel. brain volume</td>
<td>0.535**</td>
<td>0.506</td>
</tr>
<tr>
<td>Head size scaling</td>
<td>−0.859***</td>
<td>−0.780***</td>
</tr>
</tbody>
</table>

Analysis was performed either by pooling data across centres, or after mean subtraction of the mean for each centre, expressing the within centre effects. Lesion load was measured in native space, or after normalisation to standard space, expressing a relative lesion load. The number of subjects indicated only pertain to the multivariate models without brain volume parameters, since these were only available for n=335 subjects. The values indicated are the regression coefficients (*p<0.05, **p<0.01, ***p<0.001).
In the univariate analysis, alcohol consumption showed pronounced associations with increased periventricular risk (panel G), and similar but more scattered areas of association were seen for smoking (panel H). The correlation with haemoglobin concentration was mostly negative and found for areas in deep white matter (panel I).

Similarly, the concentration of low-density lipoprotein (LDL, panel J) displayed a negative correlation in an area that was much larger than the one showing positive correlation (3.7 vs 0.4 cm³, see Table 3). For the high-density fraction, the analysis showed more areas with positive rather than negative correlation (data not shown).

A history of migraine without aura was associated with a higher WMH risk in deep white matter regions lateral to the ventricles, predominantly (panel K). When migraine with aura (present in about 6%) and without aura (present in 10%) were analysed together, the total volume of significant voxels increased from 1.4 to 2.4 cm³, although in a more scattered configuration (results not shown).

Finally, panel L confirms a widespread association with a history of stroke, which is known to share part of the risk-factors associated with WMH. Spatially averaged, the presence of stroke almost doubled the odds-ratio of WMH, while—for comparison—male gender or a history of hypertension conferred a 64% increase.

Relatively small areas displayed a positive association with diabetes, while BMI showed negative associations in similar periventricular areas. Associations for COPD only reached significance in isolated voxels without any apparent pattern.

**Multivariate analyses**

As expected, the additional inclusion of the log-transformed overall volume of WMH considerably changed most patterns. The WMH-enhancing effect of age, for instance, was reduced to 20% in extent (as shown in Fig. 3, panel A), and that of hypertension was reduced to
37% of the area found in the univariate analysis (panels D–E). A pattern seemed to emerge where high blood pressure is associated with relatively less periventricular, and more deep white matter hyperintensity (turquoise vs. light-green colours in panels D–E).

In the case of the gender effect, the areas of male preponderance were diminished, while the deep white matter area showing female preponderance was clearly visualised (panel B).

The results for alcohol and smoking showed that at a given lesion load, subjects with high intake tended to have more WMH periventricularly, and less in deep white matter (panels G–H).

**Alternative visualisation**

Using the parameters estimated by any given model it is possible to construct predictions of WMH frequency as a function of each variable. By rearrangement of Eq. (1) the value of X at which P has any given value can be calculated. **Fig. 4** illustrates for both age and WMH load, the value at which P reaches 25%. For age this shows the centrifugal pattern in which hyperintensities are frequent at a relatively young age around the ventricles (ventricular caps) and progressively grows outward towards the deep white matter. The illustration for
WMH load shows the expected distribution as a function of the total amount of WMH, and confirms that in subjects with a very small load the changes are located predominantly at the anterior ventricular horns, whereas changes distant from the ventricular surface are seen on average in subjects with very large overall changes.

In order to assess the effect of entering a higher number of variables in the design matrix, a supplementary analysis was performed. Specifically, a multivariate model was constructed containing the variables Age, Gender, Presence of Hypertension and Smoking, and either containing or omitting the total lesion load as an additional covariate. The results are shown in Fig. 5 and illustrates the independent effects of Age, Gender and Hypertension. In agreement with the results presented above, all effects were diminished in the model containing Lesion Load. Due to computational constraints, inference of this analysis was not based on permutation testing of TFCE-scores, and therefore, it is not directly comparable with the results in Figs. 4 and 5.

Discussion

In the present work we used a method for probability mapping based on direct non-linear fitting of the relationship between risk factors and local probability for white matter hyperintensity (WMH). The study further confirmed that the occurrence of WMH is strongly related to age in most white matter areas, in particular those close to the ventricles. Meanwhile, other factors, such as gender, hypertension and consumption of alcohol and tobacco showed associations of similar extent but with different spatial/anatomical patterns, suggesting that the age of the subject is far from being the only determinant of WMH risk.

Distribution of WMH

The general spatial pattern of WMH predilection areas could be illustrated by considering the association with the total WMH volume (Fig. 4). As expected this is a particularly strong association, showing early (i.e. at small total WMH volumes) occurrence of WMH laterally to the anterior horns of the ventricles followed by the white matter posterior to the ventricles. At higher total volumes, extension to a more lateral deep white matter area, as well as to connecting areas occurs. While the risk distribution for most variables followed this general distribution, others considerably deviated from this pattern. Most notable was the distribution according to gender in which males showed a higher risk in most periventricular areas, but lower risk in an area corresponding to interdigitating fibres from the suprerior internal capsule and lateral corpus callosum. This was seen both with and without correction for other risk factors (as expressed by the lesion load). To our knowledge, this differential distribution has not been demonstrated before, and it is not clear from the present data whether it should be interpreted as an inherent gender difference, or as a representation of variable risk factors. It could not be demonstrated to be directly associated with any of the other risk factors investigated here.

The presence of a diagnosis of hypertension was a stronger predictor of localised WMH risk than either measured systolic or diastolic blood pressure, and among these two, diastolic pressure seemed the stronger and more robust predictor. While most of the effect of high blood pressure was abolished by correction for overall WMH volume, areas of positive association remained, especially in the deep white matter. This is in accordance with the idea that periventricular and deep white matter may be differentially affected by risk factors such as increased blood pressure.

The variables arising from self-reports of habitual alcohol and tobacco use were both strong predictors of localised WMH risk. Both effects were attenuated by correction for centre effects, and it was therefore difficult to determine, based on the present study alone, whether there may be other factors, that show covariation with e.g. alcohol consumption between centres. Because these factors have been implicated as risk factors before, we consider it likely that the associations observed in the present study also represent a direct effect of alcohol and tobacco consumption, which, for sampling reasons, happened to vary between centres. However, the effects were also attenuated by correction for the general WMH susceptibility pattern, suggesting that these risk factors contribute to a general rather than a particular, regionalised pattern.

Only small areas were showing association to the categorical risk factors: presence of diabetes or chronic obstructive pulmonary disease (COPD). Both factors are related to risk of small vessel disease and chronic hypoxia, which are commonly accepted risk factors for white matter hyperintensities. Conceivably, the limited degree of association may be due to a poor correlation between the presence of the diagnosis, and the actual severity of symptoms and long-term physiological changes. For instance, long-term markers of diabetic status, such as HbAc, were unavailable in the present study, and therefore the actual severity of illness could not be quantified. Similarly, no absolute measures of respiratory status were available to quantify the progression of COPD.

Table 3

<table>
<thead>
<tr>
<th>Model</th>
<th>Variable</th>
<th>Units</th>
<th>Volume</th>
<th>exp(β)</th>
<th>Volume</th>
<th>exp(β)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Univariate</td>
<td>Age years</td>
<td>32.5</td>
<td>0.10</td>
<td>1.36</td>
<td>0.77</td>
<td>6.6</td>
</tr>
<tr>
<td>Univariate</td>
<td>Male gender</td>
<td>Female/male = 0/1</td>
<td>16.2</td>
<td>4.10</td>
<td>1.69</td>
<td>0.61</td>
</tr>
<tr>
<td>Univariate</td>
<td>Education years</td>
<td>1.6</td>
<td>8.51</td>
<td>1.23</td>
<td>0.77</td>
<td>4.9</td>
</tr>
<tr>
<td>Univariate</td>
<td>Bp, diastolic mm Hg</td>
<td>5.0</td>
<td>0.64</td>
<td>1.02</td>
<td>0.98</td>
<td>3.5</td>
</tr>
<tr>
<td>Univariate</td>
<td>Bp, systolic mm Hg</td>
<td>3.1</td>
<td>0.21</td>
<td>1.01</td>
<td>0.99</td>
<td>2.9</td>
</tr>
<tr>
<td>Univariate</td>
<td>Hypertension No/yes = 0/1</td>
<td>18.1</td>
<td>0.18</td>
<td>1.64</td>
<td>0.64</td>
<td>6.7</td>
</tr>
<tr>
<td>Univariate</td>
<td>log(A/Alcohol)</td>
<td>log(tot. intake)</td>
<td>17.2</td>
<td>0.82</td>
<td>1.28</td>
<td>0.77</td>
</tr>
<tr>
<td>Univariate</td>
<td>log(Smoking)</td>
<td>log(pack/years)</td>
<td>12.0</td>
<td>0.75</td>
<td>1.27</td>
<td>0.82</td>
</tr>
<tr>
<td>Univariate</td>
<td>Haemoglobin g/dL</td>
<td>1.7</td>
<td>8.97</td>
<td>1.32</td>
<td>0.80</td>
<td>4.1</td>
</tr>
<tr>
<td>Univariate</td>
<td>LDL-concentration mmol/l</td>
<td>0.4</td>
<td>3.70</td>
<td>1.29</td>
<td>0.78</td>
<td>1.2</td>
</tr>
<tr>
<td>Univariate</td>
<td>Migraine No/yes = 0/1</td>
<td>1.4</td>
<td>0.68</td>
<td>1.91</td>
<td>0.46</td>
<td>0.5</td>
</tr>
<tr>
<td>Univariate</td>
<td>Stroke No/yes = 0/1</td>
<td>36.0</td>
<td>0.06</td>
<td>1.98</td>
<td>0.55</td>
<td>5.5</td>
</tr>
<tr>
<td>Univariate</td>
<td>BMI kg/m2</td>
<td>1.0</td>
<td>4.31</td>
<td>1.27</td>
<td>0.80</td>
<td>2.0</td>
</tr>
<tr>
<td>Univariate</td>
<td>Diabetes No/yes = 0/1</td>
<td>3.1</td>
<td>0.75</td>
<td>1.96</td>
<td>0.45</td>
<td>2.2</td>
</tr>
<tr>
<td>Univariate</td>
<td>COPD No/yes = 0/1</td>
<td>0.8</td>
<td>0.76</td>
<td>2.12</td>
<td>0.30</td>
<td>0.9</td>
</tr>
</tbody>
</table>

For each model the total volume (cm$^3$) of significantly correlated voxels is given, as well as the median value of the parameter estimate within that volume. The numbers in the shaded columns refer to areas with a positive association (increasing risk of WMH), the un-shaded columns to areas where higher values of the parameter of interest are associated with lower WMH risk.

The parameter estimate is given as $\exp(\beta)$, i.e. the increase in odds ratio at a unit increase in the independent variable. Abbreviations: BPDIA (diastolic blood pressure), BPSYS (systolic blood pressure), BMI (body mass index), HGB (haemoglobin concentration), COPD (chronic obstructive pulmonary disease).
Although not significant in the analysis of global lesion load, the voxel-based analysis demonstrated an association between the concentration of LDL and WMH-probability. In the univariate analysis the association was negative, and it remained predominantly negative after correction for overall lesion load. This is in agreement with previous findings that have demonstrated higher cholesterol levels in stroke patients with low WMH load (Khan et al., 2007), and lower WMH load in patients with hypercholesterolemia (Jimenez-Conde et al., 2010) suggesting a protective role for hyperlipidemia. In our study the inclusion was not based on the presence of previous stroke, and the results may add further support to the notion that the effect of blood lipids in the two studies mentioned is not due to a bias of including only patients with symptomatic ischemic lesions.

Associations between WMH and migraine both with and without aura have previously been demonstrated (Kruit, 2004), but a voxel-based analysis of the spatial distribution has not been provided before. Our results differ slightly from those of Kruit et al. in demonstrating the association in a group consisting of both men and women, however, we did not test specifically for an interaction between gender and migraine. The present study did not include a detailed clinical history of
migraine symptomatology and treatment, something that should be considered in future research.

Possible limitations

The way the subjects were recruited presents a potential limitation of the study. All subjects were recruited because of some contact with the hospital system, and although they did not have overt neurological or psychiatric disease, they are likely to represent a group with an increased risk for functional deterioration, compared to the background population. The significant effects found are thus only valid within this group, and likely to be underestimated relative to what would have been found in a comparison with a WMH free background population. Furthermore, the cross-sectional nature of the study precluded a more detailed analysis of the spatial patterns of WMH growth, which is likely to be different between punctate and confluent lesions (Schmidt et al., 2003).

The full dataset was not available for all subjects, and as seen in Table 1 there were more missing data for laboratory values, than for primary values such as age, gender and blood pressure, and this represents a potential confound regarding the volume of activation. However, we repeated all analyses for the set of subjects in which laboratory measurements were present, and found virtually unchanged results. Although, in the data presented above, we decided to maintain the highest power for each variable, we could thus ascertain that any differences in effect size (volume of association) were not just due to differences in statistical power.

In the current study we present results from both univariate analyses and analyses with correction for overall lesion load. The results differ considerably in many regions, because lesion load represents the sum of all (known or unknown) risk factors, and thus, the overall propensity for WMH. The residual areas of significance therefore represent the differential effect of a particular covariate. A different way of addressing the potential problem of colinearity is to apply a
multivariate model. This analysis showed remaining effects of Age, Gender and Hypertension, while Smoking effects seemed quite attenuated. It cannot be ruled out that this finding to some extent may reflect colinearity, as smoking was indeed more prevalent among men than women. However, the results are not directly comparable to the lesion load corrected maps in Fig. 2, which are constructed from permutation-testing of TFCF-scores, rather than voxel-wise thresholding based on conventional distributional assumptions.

It should be noted that while the conventional analysis of lesion load failed to show any association with diabetes, hyperlipidemia and others, such associations were indeed demonstrated using the voxel-based approach. This suggests that the voxel-based analysis may have a higher sensitivity, especially in cases where the association affects only a small area, or displays a pattern of both positive and negative effects.

In the present study we considered risk factors, rather than symptom-lesion mapping. Future studies might address the correlation between specific symptoms and the spatial localisation of WMH, however, such studies should consider the large spatial covariance of the WMH distribution, which may limit the association with more detailed parameters of cognitive or motor function.

Conclusion

In conclusion we have demonstrated significant associations patterns between WMH and a number of risk factors, using a rigorous voxel-based method. Gender, hypertension and smoking were among the risk factors giving the most distinct patterns. We have demonstrated the capability of the method to produce normative spatial probability maps for WMH as a function of given risk factors. We suggest that future studies analyse the association with risk factors and/or functional consequences in more detail, while striving to reconcile the need for large study populations and minimisation of centre effects.

Disclosure statement

None of the authors state any actual or potential conflicts of interest with people or organisations within 3 years of beginning the present work.

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Appendix A. List of participating centres and personnel

Helsinki, Finland (Memory Research Unit, Department of Clinical Neurosciences, Helsinki University): Timo Erkinjuntti, MD, PhD, Tarja Pohjasvaara, MD, PhD, Pia Pihlaja, MD, Raija Vilkkoski, PhD, Hanna Jokinen, PhD, Mei-Ja-Marij Somerkoski, MPSych, Riitta Mäntylä, MD, PhD, Olli Salonen, MD, PhD; Graz, Austria (Department of Neurology and Department of Radiology, Division of Neuroradiology, Medical University Graz): Franz Fazekas, MD, Reinhold Schmidt, MD, Stefan Ropele, PhD, Brigitte Rous, MD, Katja Petrovic, MagPsychol, Ulrike Garmezi, Alexandra Seewann, MD; Lisboa, Portugal (Serviço de Neurologia, Centro de Estudos Egas Moniz, Hospital de Santa Maria): José M. Ferro, MD, PhD, Ana Verdelho, MD, Sofia Madureira, PsyD, Carla Moleiro, PhD; Amsterdam, The Netherlands (Department of Radiology and Neurology, VU Medical Center): Philip Scheltens, MD, PhD, Ilse van Straaten, MD, Frederik Barkhof, MD, PhD, Alida Gouw, MD, Wiesje van der Flier, PhD; Göteborg, Sweden (Institute of Clinical Neuroscience, Goteborg University): Anders Wallin, MD, PhD, Michael Jonsson, MD, Karin Lind, MD, Arto Nordlund, PsyD, Sindre Rolstad, PsyD, Ingela Isblad, RN; Huddinge, Sweden (Karolinska Institutet, Department of Neurobiology, Care Sciences and Society; Karolinska University Hospital Huddinge): Lars-Olof Waldemar, MD, PhD, Milvia Crisby, MD, PhD, Anna Pettersson, RPT, PhD, Karina Amberla, PsyD; Paris, France (Department of Neurology, Hospital Lariboisiere): Hugues Chabrier, MD, PhD, Karen Hernandez, psychologist, Annie Kurtz, psychologist, Dominique Hervé, MD, Sarah Benisty, MD, Jean Pierre Guichard, MD; Mannheim, Germany (Department of Neurology, University of Heidelberg, Klinikum Mannheim): Michael Henninger, MD, Christian Blahak, MD, Hansjörg Baezner, MD, Martin Wiarda, PsyD, Susanne Seip, RN; Copenhagen, Denmark (Memory Disorders Research Group, Department of Neurology, Rigshospitalet, and the Danish Research Center for Magnetic Resonance, Hvidovre Hospital, Copenhagen University Hospitals): Gunhild Waldemar, MD, DMSc, Egil Rostrup, MD, MSc; Charlotte Ryberg, MSc, Tim Dyrbø MSc, Olaf B. Paulson, MD, DMSc; Ellen Garde, MD, PhD; Newcastle-upon-Tyne, UK (Institute for Ageing and Health, Newcastle University): John O’Brien, DM, Sanjeet Pakrasi, MRCPsych, Mani Krishnan MRCPsych, Andrew Teodorczuk, MRCPsych, Michael Fibrank, PhD, Philip English, DCR, Thais Minett, MD, PhD.

The Coordinating centre is in Florence, Italy (Department of Neurological and Psychiatric Sciences, University of Florence: Domenico Inzitari, MD (Study Coordinator); Luciano Bartolini, PhD, Anna Maria Basile, MD, PhD, Eliana Magnani, MD, Monica Martini, MD, Mario Mascalchi, MD, PhD, Marco Moretti, MD, Leonardo Puntori, MD, PhD, Anna Poggesi, MD, Giovanni Pracucci, MD, Emilie Salvadori, PhD, Michela Simon, MD.

The LADIS Steering Committee is formed by Domenico Inzitari, MD (study coordinator), Timo Erkinjuntti, MD, PhD, Philip Scheltens, MD, PhD, Marieke Visser, MD, PhD, and Peter Langhorne, MD, BSc, PhD, FRCP who replaced in this role Kjell Asplund, MD, PhD beginning with 2005.

References


