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Classification and characterization of periventricular and deep white matter hyperintensities on MRI: A study in older adults

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A B S T R A C T

White matter hyperintensities (WMH) are frequently divided into periventricular (PWMH) and deep (DWMH), and the two classes have been associated with different cognitive, microstructural, and clinical correlates. However, although this distinction is widely used in visual ratings scales, how to best anatomically define the two classes is still disputed. In fact, the methods used to define PWMH and DWMH vary significantly between studies, making results difficult to compare. The purpose of this study was twofold: first, to compare four current criteria used to define PWMH and DWMH in a cohort of healthy older adults (mean age: 69.58 ± 5.33 years) by quantifying possible differences in terms of estimated volumes; second, to explore associations between the two WMH sub-classes with cognition, tissue microstructure and cardiovascular risk factors, analysing the impact of different criteria on the specific associations. Our results suggest that the classification criterion used for the definition of PWMH and DWMH should not be considered a major obstacle for the comparison of different studies. We observed that higher PWMH load is associated with reduced cognitive function, higher mean arterial pressure and age. Higher DWMH load is associated with higher body mass index. PWMH have lower fractional anisotropy than DWMH, which also have more heterogeneous microstructure. These findings support the hypothesis that PWMH and DWMH are different entities and that their distinction can provide useful information about healthy and pathological aging processes.

Introduction

White matter hyperintensities of presumed vascular origin (WMH), also called white matter lesions or leukoaraiosis (Wardlaw et al., 2013), are divided into periventricular (PWMH) and deep (DWMH) (De Groot et al., 2002; Kim et al., 2008). This distinction is at the base of most visual rating scales used in clinical settings (Fazekas et al., 1987; Scheltens et al., 1993) and seems to reflect different functional, histopathological, and aetiological features (Debette and Markus, 2010; Kim et al., 2008). However, the anatomical definition of the two classes is somewhat arbitrary (Barkhof and Scheltens, 2006; DeCarli et al., 2005; Sachdev and Wen, 2005) and varies across studies, potentially contributing to inconsistencies in results and making comparisons difficult.

Among the criteria used to define PWMH and DWMH, one widely accepted measurement follows the “continuity to ventricle” rule, according to which PWMH are WMH contiguous with the margins of each lateral ventricle, while DWMH are WMH separate from the ventricles (Fazekas et al., 1987; Fazekas et al., 1993; van den Heuvel et al., 2006). Other criteria use the distance from the ventricles to define the two classes, defining PWMH as WMH within an arbitrary distance from the ventricle surface, most frequently 10 mm (DeCarli et al., 2005), or an empirical distance (Wen and Sachdev, 2004). Another suggested classification further divides WMH into four categories: juxtaventricular (within 3 mm from the ventricle surface), periventricular (between 3 and 13 mm), deep and juxtacortical WMHs (within 4 mm from the corticomedullary junction) (Kim et al., 2008). To the best of our knowledge, these different criteria adopted to classify PWMH and DWMH have not been quantitatively compared within a single analytic setting.

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Kim and colleagues reviewed the differences between PWMH and DWMH and concluded that they have different functional, histopathological and aetiological correlates (Kim et al., 2008). They reported a prevalent link between PWMHs and impaired cognitive performance in older adults, although this was not consistent across studies (Bolandzadeh et al., 2012). DWMH seem to be instead preferentially associated with mood disorders (de Groot et al., 2000; Kim et al., 2008; Krishnan et al., 2006). Postmortem studies showed that PWMH have discontinuous ependyma (and therefore high extracellular fluid content), gliosis, loosening of the white matter fibers, and myelin loss. DWMH show more axonal loss, vacuolation and increased tissue loss in more severe lesions, suggesting infarction, in addition to demyelination and gliosis (Kim et al., 2008; Schmidt et al., 2011; Wardlaw et al., 2015). Since there are only few pathology studies, the use of neuroimaging methods such as diffusion tensor imaging (DTI) MRI offers the potential of detecting *in vivo* microstructural changes in the integrity of the brain's white matter (Wardlaw et al., 2015). In fact, it has been shown that PWMH have increased axial diffusivity (AD) and radial diffusivity (RD) relative to normal appearing WM (Bastin et al., 2009). However, they did not include DWMH in their evaluation. Furthermore, PWMHs and DWMHs have been associated with different cardiovascular risk factors suggesting at least partially distinct pathogenic mechanisms. In their review, Kim and colleagues (Kim et al., 2008) concluded that smooth PWMH may be linked to the increase of interstitial fluid (cerebrospinal fluid leakage), irregular PWMH are more likely determined by chronic hemodynamic insufficiency (hypoperfusion), whereas DWMH may be more attributed to small vessel disease.

All these associations support the notion that the subdivision into PWMH and DWMH is clinically relevant. However, there is currently no objective, unambiguous and non-disputable approach to sharply divide PWMH and DWMH (Barkhof and Scheltens, 2006; DeCarli et al., 2005; Sachdev and Wen, 2005).

The aim of our study was twofold: first, to quantitatively compare the most common criteria used in the literature for the classification of PWMH and DWMH by estimating differences in respective resulting WMH volumes; second, to replicate previously reported associations between WMH and cognitive correlates, tissue microstructure (using DTI-derived metrics) and cardiovascular risk factors (potentially involved in WMH aetiology). Specifically, we analysed how associations with these cognitive, microstructural and clinical correlates (cardiovascular risk factors) were influenced by the different classification criteria, with a view to establishing the most robust method of differentiating PWMH from DWMH. Ultimately, we aim to understand the cause of possible contrasting results in the literature and give further insight into the potential clinical utility of WMH sub-classifications.

We performed this quantitative evaluation of the cognitive, microstructural and clinical correlates of PWMH and DWMH on the Whitehall imaging sub-study cohort (Filippini et al., 2014), which represents an ideal sample for the different aims of this study due to the richness of imaging and non-imaging data.

Methods

Subjects and MRI data acquisition

The sample was drawn from 563 participants recruited to take part in the Whitehall II Imaging Sub-Study between April 2012 and December 2014 (Filippini et al., 2014). The Whitehall II Study is a prospective occupational cohort study established in 1985 (Marmot and Brunner, 2005). Ethical approval was obtained from the University of Oxford Central University Research Ethics Committee, and the UCL Medical School Committee on the Ethics of Human Research. Informed written consent was obtained from all participants.

Thirty seven subjects were excluded: 14 because of the presence of major lesions (tumours or major strokes), diagnosis of multiple sclerosis or hydrocephalus, and 24 because of incomplete MRI scans or the presence of excessive motion or other artefacts in the images. Data from the remaining 525 subjects (mean age 69.58 ± 5.33 years) entered into subsequent analyses.

MRI data were acquired at the Oxford Centre for Functional MRI of the Brain (FMRIB) using a 3-Tesla, Siemens Magnetom Verio scanner with 32-channel head coil. Details of acquisition sequences and preprocessing have been described previously (Filippini et al., 2014). In this study we used high-resolution T1 images, FLAIR images and diffusion weighted images, in particular the DTI-derived maps of fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD), generated by fitting a diffusion tensor model at each voxel.

Segmentation of WMH and PWMH/DWMH classification criteria

WMH were automatically segmented on FLAIR images with a newly developed tool, BIANCA (Brain Intensity AbNormality Classification Algorithm), a fully-automated, supervised method for WMH detection, based on the k-nearest neighbour algorithm (Griffanti et al., 2016). Briefly, BIANCA classifies the image voxels based on their intensity and spatial features, where the intensity features were extracted from FLAIR, T1 and FA images (additional options used: local average intensity within a kernel of size = 3 voxels; MNI coordinates as spatial features, with a weighting factor of 2; 24 manually segmented images as training dataset). The output image represents the probability per voxel of being WMH. The WMH maps used for further analyses in this work included voxels exceeding a probability of 0.9 of being WMH and located within a white matter mask automatically generated from T1-weighted images, excluding cortical grey matter, cerebellum, brainstem and subcortical structures (see Griffanti et al., 2016 for further details). The WMH map would also include the possible small hyperintense rim around infarct-like lesions (lacunes) (Wardlaw et al., 2013).

In order to apply the sub-classification criteria, lateral ventricles were segmented from T1 images. Tissue segmentation was performed with FSL-FAST, then ventricles were extracted from the cerebrospinal fluid (CSF) map as the clusters with the greatest overlap with a registered and dilated version of the lateral ventricles of the Harvard-Oxford atlas. The obtained ventricles mask was then registered in FLAIR space.

WMH were then further sub-classified into PWMH and DWMH and the relative volumes were calculated according to the following 4 criteria (Fig. 1):

1. Continuity to ventricles (Fazekas et al., 1987; Fazekas et al., 1993; van den Heuvel et al., 2006): a WMH adjacent to the ventricle surface is PWMH, otherwise it is DWMH. Each cluster in the WMH map was considered PWMH if it was, at least partially, overlapping with a dilated version of the ventricles mask.
2. 10 mm distance (DeCarli et al., 2005): a WMH less than 10 mm distance from the ventricles is PWMH, otherwise it is DWMH. Each voxel in the WMH map was considered PWMH if it was within 10 mm distance from the ventricles (calculated as the geometrical distance from the ventricle mask using the FSL command *distancemap*).
3. 3–13 mm distance (Kim et al., 2008): a lesion less than 3 mm from the ventricles is classified as juxtaventricular; a lesion between 3 and 13mm distance from the ventricles is periventricular, otherwise it is DWMH. Since BIANCA is not currently optimized to detect juxtacortical WMH, this further sub-classification of DWMH was not performed.
4. Empirical distance: Wen and Sachdev (Wen and Sachdev, 2004) defined the periventricular region empirically. After manually measuring the width of the rims and caps in 477 healthy 60- to 64-year-

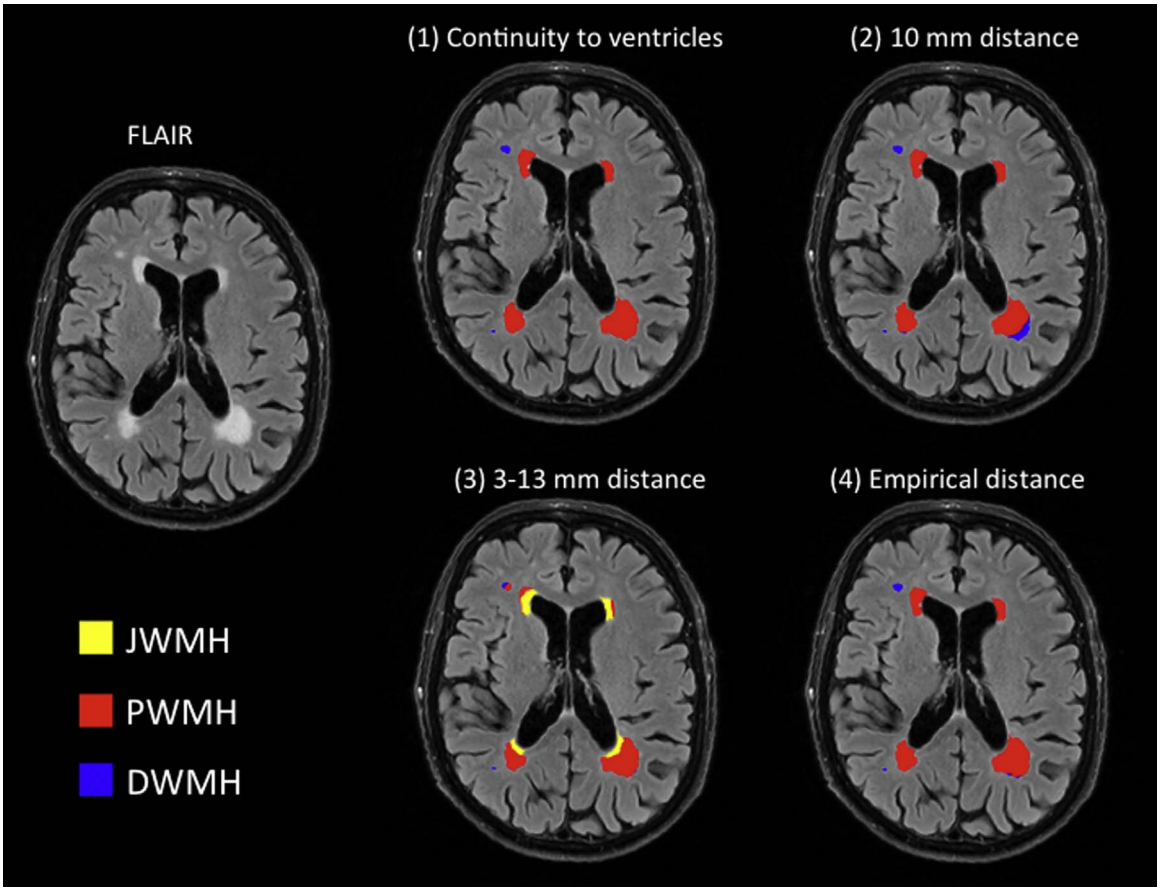

Fig. 1. Example of WMH sub-classification obtained with the four criteria compared in this study (see main text for details). Legend: JWMH = juxtaventricular white matter hyperintensities; PWMH = periventricular white matter hyperintensities; DWMH = deep white matter hyperintensities.

old individuals, they plotted the values and defined the periventricular width for the rims, anterior and posterior caps as the width that included 95% of the sample. The resulting map of their study was used to extract PWMH and DWMH in our study, by registering the map to the single subject's space and extracting the values for the two WMH classes.

The resulting volumes of PWMH and DWMH were log-transformed and entered into subsequent analyses. The comparison of the volumes across the four criteria was performed using a one-way ANOVA for repeated measures with post-hoc Bonferroni correction for multiple comparisons.

Cognitive, microstructural and clinical correlates of PWMH/DWMH

The following measures, collected at the time of the MRI scan, were selected as indices of executive function, processing speed, and memory, in line with (Bolandzadeh et al., 2012): Hopkins Verbal Learning Test (HVLT, immediate and delayed recall), Trail Making Test (TMT), letter and category fluencies, Boston Naming-60, digit span forward, backward and sequence, digit symbol and digit coding. For details about the tests used, please refer to (Filippini et al., 2014). These measures were entered into a regression analysis as possible predictors of PWMH and DWMH volumes (dependent variable). The model also included sex, education (number of years of uninterrupted full time education) and total brain volume (calculated as the sum of white matter, grey matter and CSF volumes derived from tissue segmentation using FSL-FAST) as covariates, while age was included among the potential risk factors (however, for completeness, we also repeated the analyses with age as covariate). All independent variables were entered into the model at the same time (enter method or forced entry).

To test the association with mood disorders, volumes of PWMH and DWMH were correlated with current depressive symptoms, measured at the time of the MRI scan using the Centre for Epidemiological Studies Depression Scale (CES-D), a clinically validated self-report questionnaire. 524 participants (the CES-D score was missing for one subject) were categorized into control and sub-threshold depression groups based on their CES-D scores (score greater than 10 was used to define sub-threshold depression, as in (Allan et al., 2016; Tudorascu et al., 2014)) and the volumes of PWMH and DWMH were compared between the two groups using an independent t-test. The CES-D score was also considered as a continuous variable across all participants and correlated with PWMH and DWMH volumes (correcting for age, sex, and total brain volume).

In the absence of histopathological data, we studied WMH microstructure using DTI-derived measures. Accordingly, we compared PWMH and DWMH in terms of average FA, MD, AD, RD within the WMH using paired t-tests (or one-way ANOVA for repeated measures with post-hoc Bonferroni correction for criterion 3) to evaluate potential differences in underlying microstructure between PWMH and DWMH.

Finally, to test the link between WMH and cardiovascular risk factors, we performed a regression analysis including age, Framingham stroke 10-year percentage risk (i.e. the probability of stroke in the next 10 years, calculated from age, sex, smoking habits, diabetes mellitus, systolic blood pressure, prior cardiovascular disease, atrial fibrillation, left ventricular hypertrophy and use of hypertensive medications (D'Agostino et al., 1994)), mean arterial pressure (MAP, calculated as (systolic blood pressure + 2*diastolic blood pressure)/3), and body mass index (BMI). The model included sex and total brain volume as covariates. All independent variables entered in the model at the same time.

Table 1
Sample characteristics.

Descriptive Statistics		N	Minimum	Maximum	Mean	Std. Dev.
Demographic						
Age [years]		525	60.30	83.00	69.58	5.33
Sex [M:F]		424:101				
Education [years]		525	6	23	14.03	3.08
Functional correlates (cognition and mood)						
Letter fluency		525	3	31	15.75	4.55
Categorical fluency		525	3	40	22.11	5.63
TMT A		523	13	125	30.74	11.91
TMT B		522	24	321	67.98	36.36
TMT B-A		518	0	253	37.74	30.93
Boston Naming-60		525	15	60	57.27	4.49
HVLT immediate recall		525	10	36	27.51	4.74
HVLT delayed recall		525	0	12	9.27	2.68
Digit Span Forward		525	5	16	10.98	2.28
Digit Span Backward		525	4	16	9.76	2.54
Digit Span Sequence		525	0	16	10.12	2.49
Digit Symbol Total		525	13	46	30.86	5.82
Digit Coding		524	13	112	62.44	13.31
CES-D total score		524	0	39	5.23	6.14
Sub-threshold depression (CES-D > 10) [NO:YES]		441:84				
Cardiovascular risk factors						
MAP		522	68.33	146.67	97.80	11.70
BMI		525	14.30	42.40	26.20	4.19
Framingham 10-years risk score		525	2	84	11.60	8.31
MRI measures						
Total brain volume [mm^3]		525	1023552.00	1927776.00	1441226.86	129398.90
Total WMH volume [mm^3]		525	1103.91	26085.64	5972.62	3850.15
WMH sub-classes volumes [mm^3]						
	P(1)	525	576.60	25394.94	4737.78	3585.96
	D(1)	525	122.41	7330.79	1234.84	1059.45
	P(2)	525	710.09	23885.03	4691.42	3160.19
	D(2)	525	58.71	13013.74	1281.19	1290.78
	J(3)	525	521.77	8462.95	2780.68	1278.37
	P(3)	525	146.23	19237.30	2231.91	2512.92
	D(3)	525	28.80	9327.02	960.03	955.41
	P(4)	525	670.21	22123.10	4418.52	2943.56
	D(4)	525	125.73	11459.52	1554.09	1381.40

Legend: TMT = Trail Making Test; HLVT = Hopkins Verbal Learning Test; CES-D = Centre for Epidemiological Studies Depression Scale; MAP = mean arterial pressure; BMI = body mass index; WMH = white matter hyperintensities; P = PWMH volume; J = JWMH volume; D = DWMH volume; 1–4: criteria described in the methods section.

All the tests were repeated using the four classification criteria described above.

Results

Summary descriptive statistics (mean, standard deviation and range) of the sample characteristics are reported in Table 1, while supplementary Figure S1 illustrates the spatial distribution of WMH in the sample.

Fig. 2 shows the volumes calculated with different criteria (see Table 1 for details). The repeated measures ANOVA determined that PWMH and DWMH volumes differ significantly among criteria (PWMH: $F_{(1.184,\ 620.386)} = 1404.663$, $p < 0.001$; DWMH: $F_{(2.092,\ 1096.122)} = 258.717$, $p < 0.001$). Post-hoc tests using Bonferroni correction revealed that DWMH calculated with the continuity to ventricles rule and the 10 mm distance rule are comparable, while other volumes are significantly different across criteria (see supplementary Table S1 for details). The 3–13 mm distance rule was different from the other methods by definition, but the differences with respect to the other criteria remained significant also when considering JWMH and PWMH together.

Results of the regression analysis exploring the functional correlates of WMH are reported in Table 2. A significant model emerged for PWMH (and JWMH) regardless of which criterion was used. Among the functional measures, Digit Span forward was a significant predictor variable for PWMH (with criteria 1,2,4) and JWMH. The Trail Making Test was an additional significant predictor for JWMH. For DWMH, only the empirical distance rule yielded a significant model in which only total brain volume was a significant predictor for DWMH.

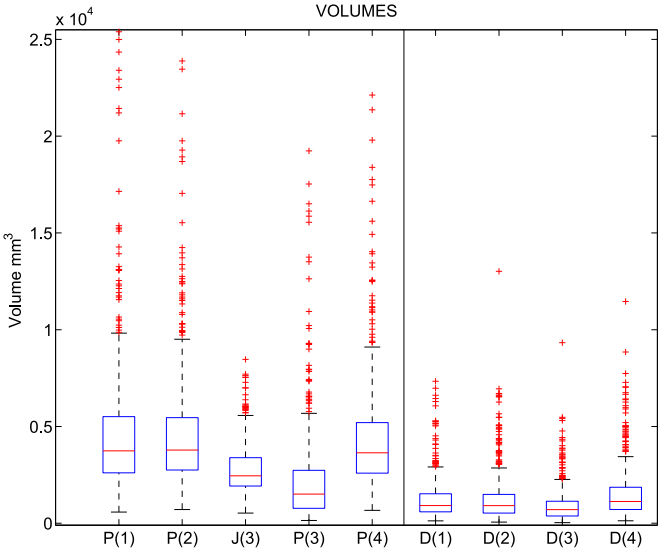

Fig. 2. Comparison of currently used criteria to classify PWMH and DWMH. Boxplot of volumes for PWMH (P), JWMH (J) and DWMH (D), calculated with the four criteria described in the methods section.

Among the covariates, brain volume was the only significant

Table 2
Functional correlates of WMH. Regression analysis results.

Volume	Model			Significant predictors	
	F(14,503)	p-value	Adjusted R square		
P(1)	5.716	< 0.001	0.113	DS forward total brain volume	p=0.012, beta=−0.127 p < 0.001, beta=0.309
P(2)	5.997	< 0.001	0.119	DS forward (DCOD) total brain volume	p=0.011, beta=−0.127 (p=0.069, beta=−0.100) p < 0.001, beta=0.307
J(3)	8.236	< 0.001	0.164	TMT A DS forward total brain volume	p=0.019, beta=0.121 p=0.002, beta=−0.148 p < 0.001, beta=0.382
P(3)	2.933	< 0.001	0.050	(DCOD) total brain volume	(p=0.057, beta=−0.108) p < 0.001, beta=0.190
P(4)	5.189	< 0.001	0.102	DS forward total brain volume	p=0.017, beta=−0.120 p < 0.001, beta=0.284
D(4)	2.388	0.003	0.036	total brain volume (sex)	p < 0.001, beta=0.243 (p= 0.068, beta=−0.095)

Factors included in the model: Hopkins Verbal Learning Test (HVLT, immediate and delayed recall), Trail Making Test (TMT A, B and B-A), Letter fluency, category fluencies, Boston Naming-60, Digit span (DS) forward, backwards and sequence, Digit symbol and Digit coding (DCOD), total brain volume, sex, education. Legend: P = PWMH volume; J = JWMH volume; D = DWMH volume; 1–4: criteria described in the methods section. Predictors showing a trend of significance ($0.05 < p < 0.07$) are reported in brackets.

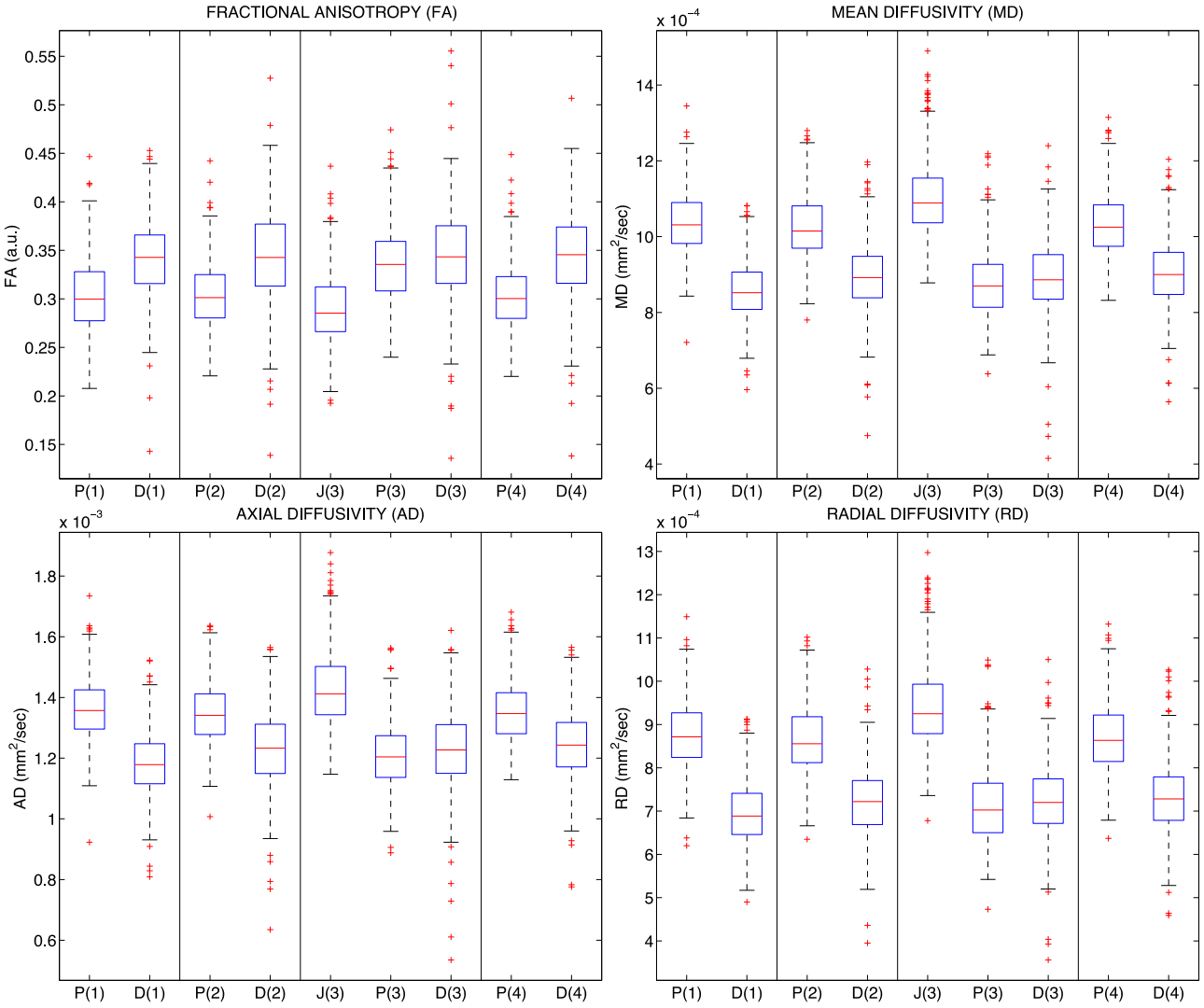

Fig. 3. Microstructural characteristics of PWMH and DWMH. The boxplots show DTI-derived measures calculated within PWMH (P), JWMH (J) and DWMH (D), using the four criteria described in the methods section.

predictor. Including age in the model did not alter the relationships between functional correlates and PWMH and DWMH (the model remained significant for PWMH with all criteria, for JWMH, and for DWMH only with the empirical distance rule).

There were no significant differences between the control and sub-threshold depression groups in PWMH or DWMH volumes calculated with any criterion. Also the correlation between WMH volumes and CES-D score was not significant (details are reported in supplementary Table S2).

Fig. 3 shows the results of the comparison between the WMH sub-classes in terms of DTI-derived measures. When using any of the 2-class criteria (i.e. (1) continuity to ventricles, (2) 10 mm and (4) empirical distance), the PWMH showed significantly lower FA, and higher MD, AD and RD than DWMH ($p < 0.001$, with any criterion for all measures). When using the 3–13 mm distance rule (3) leading to three WMH sub-classes, the JWMH had significantly lower FA than PWMH ($p_{corr} < 0.001$), which had lower FA than DWMH ($p_{corr} < 0.001$), suggesting that FA increases with the distance from the ventricles (JWMH < PWMH < DWMH). When testing the other DTI-derived measures, JWMH showed significantly higher MD, AD and RD values relative to DWMH and PWMH ($p_{corr} < 0.001$, for all comparisons). PWMH and DWMH were more similar, although PWMH had significantly lower MD ($p_{corr}=0.001$), AD ($p_{corr}=0.001$), and RD ($p_{corr}=0.004$) than DWMH (JWMH > > DWMH > PWMH).

Results of the regression analyses exploring association with cardiovascular risk factors are reported in Table 3. Using the enter method, a significant model emerged both for PWMH (and JWMH) and for DWMH, calculated with all of the four criteria, but the significant predictor variables were different for the two classes. Mean arterial pressure was a significant predictor for PWMH with all criteria and for JWMH, but for DWMH only with the empirical distance criterion. On the other hand, body mass index was significant predictor for DWMH, but for PWMH only with the 3-class criterion.

Age was a significant predictor for PWMH (and JWMH) calculated with all the criteria ($p < 0.001$) and for DWMH calculated with the empirical distance rule ($p=0.001$), but the effect size (beta) was higher for PWMH than for DWMH. Among the covariates, brain volume was a significant predictor in all models except for D(1), while sex was significant predictor for P(2), J(3) and P(4).

Discussion

In this study we compared the most widely used criteria for the quantification of PWMH and DWMH and examined functional, microstructural and clinical correlates of the two WMH sub-classes. This was done with the ultimate aim of helping to compare studies, replicate previous results, and understand the cause of possible contrasting results in the literature.

When comparing the WMH volumes calculated with four criteria for classification of PWMH and DWMH, our results showed that the continuity to ventricles and 10 mm distance rules give the most similar results in terms of WMH volume quantification. These are the two most widely used criteria in the literature, not only in volumetric methods for WMH measurement but also in visual rating scales. Our results suggest that studies using either of the two methods should be comparable. However, it needs to be kept in mind that, when using

Table 3
Clinical correlates of WMH. Regression analysis results.

Volume	Model			Significant predictors	
	F(6,515)	p-value	Adjusted R square		
P(1)	25.071	< 0.001	0.217	MAP	p=0.012, beta=0.108
				Age	p < 0.001, beta=0.354
				total brain volume	p < 0.001, beta=0.289
				(sex)	(p=0.055, beta=−0.088)
D(1)	2.488	0.022	0.017	BMI	p=0.004, beta=0.131
P(2)	26.628	< 0.001	0.228	MAP	p=0.009, beta=0.113
				Age	p < 0.001, beta=0.363
				total brain volume	p < 0.001, beta=0.286
				sex	p=0.040, beta=−0.093
D(2)	3.889	0.001	0.032	BMI	p=0.008, beta=0.118
				(Age)	(p=0.060, beta=0.100)
				total brain volume	p=0.009, beta=0.131
J(3)	28.068	< 0.001	0.238	MAP	p=0.021, beta=0.098
				Age	p < 0.001, beta=0.319
				total brain volume	p < 0.001, beta=0.368
				sex	p=0.030, beta=−0.098
P(3)	16.555	< 0.001	0.152	MAP	p=0.018, beta=0.106
				BMI	p=0.049, beta=0.082
				Age	p < 0.001, beta=0.330
				total brain volume	p < 0.001, beta=0.167
D(3)	3.173	0.005	0.024	BMI	p=0.031, beta=0.096
				Framingham	p=0.035, beta=−0.122
				total brain volume	p=0.015, beta=0.123
P(4)	23.215	< 0.001	0.204	MAP	p=0.007, beta=0.116
				Age	p < 0.001, beta=0.351
				total brain volume	p < 0.001, beta=0.267
				sex	p=0.026, beta=−0.103
D(4)	9.477	< 0.001	0.089	BMI	p < 0.001, beta=0.164
				MAP	p=0.046, beta=0.093
				Age	p=0.001, beta=0.175
				total brain volume	p < 0.001, beta=0.216

Factors included in the model: Framingham score, age, mean arterial pressure (MAP), body mass index (BMI), sex, total brain volume. Legend: P = PWMH volume; J = JWMH volume; D = DWMH volume; 1–4: criteria described in the methods section. Predictors showing a trend of significance ($0.05 < p < 0.07$) are reported in brackets.

visual rating scales, the intra-rater variability is still a source of heterogeneity in WMH quantification. When quantifying the WMH load in terms of volumes, the 10 mm distance rule should be more suitable for automatic implementation, since confluent lesions that are touching the ventricles but expanding in the deep WM would be probably considered as separate entities by a human rater, even when adopting the continuity to ventricles rule.

Using the map created with the empirical distance rule we obtained significantly smaller volumes of PWMH (and higher DWMH) compared to the other (2-class) methods. This difference suggests that this criterion might be sample specific and using this rule might lead to results that are not comparable across studies.

In agreement with literature (Bolandzadeh et al., 2012; Kim et al., 2008), we found PWMH, but not DWMH, to be associated with impaired cognitive function. This result seems to remain consistent despite different MRI sequences, WMH quantification methods, and neuropsychological batteries used. In particular, the PWMH were associated with working memory. This is in line with the notion that executive functions rely on long distance connections in the white matter, which are more likely to be disrupted by PWMH. In contrast, DWMH are believed to primarily disrupt short connections (Bolandzadeh et al., 2012).

We did not find differences between controls and sub-threshold depression groups, nor significant correlation between WMH volumes and CES-D score, as a measure of mood disorders (de Groot et al., 2000; Krishnan et al., 2006). This could be due to the fact that the variability in our sample is quite low, with very few subjects showing depression symptoms (i.e. only 38 out of 524 participants scored more than 16).

The DTI findings suggest that WMH classes have different underlying microstructural properties. When using any of the two-class criteria, PWMH showed significantly decreased FA and increased MD, AD and RD with respect to DWMH, supporting the neuropathological observation that PWMH are characterized by gliosis, loosening of the white matter fibers, and myelin loss. DWMH instead are more heterogeneous and may present less gliosis, but more axonal loss, vacuolisation and arteriolosclerosis (Wharton et al., 2015). When further subdividing into 3 classes, JWMH had the lowest FA and highest MD, AD and RD with a significant difference from PWMH, suggesting that the microstructure in closer proximity to the ventricles is substantially different in nature from parenchymal WMH, possibly reflecting subependimal gliosis (Kim et al., 2008; Wharton et al., 2015). With this criterion, the DWMH had greater mean magnitude of water diffusion (higher MD), possibly representing greater axonal (higher AD) and myelin (higher RD) damage, than PWMH. These differences may be explained by the fact that PWMH are characterized by more microgliosis, which may selectively "pseudo-normalise" MD. Further studies should explore the above hypotheses in more detail and clarify whether the first lining around the ventricles should not even be considered WMH of vascular origin.

We also found that PWMH and DWMH have distinct associations with cardiovascular risk factors, suggesting that they may have different aetiology. More precisely, PWMH were significantly associated with arterial pressure but not with BMI. On the contrary, DWMH were associated with BMI but not with arterial pressure. This dissociation is difficult to interpret in light of previous pathology studies that have hypothesized that deep WMH changes (DWMH) present more hypoxic/ischemic damage whereas periventricular changes may have a greater inflammatory/metabolic component (Fazekas et al., 1993; Fernando et al., 2006). Since we only had a limited set of cardiovascular risk factors to be entered in the model, our findings should be interpreted cautiously and prompt further studies, which we hope will benefit from the methodological suggestions of the present study. Age was a significant predictor mainly for PWMH, in line with studies suggesting that PWMH are specifically linked to age-related neurodegenerative processes.

The finding that total brain volume was a significant predictor of both PWMH and DWMH suggests that is important to take into account brain size when quantifying WMH, as it may influence the associations of interest. This can be done, for example, by adding brain volume as a covariate or normalizing the volumes.

Taken together, these results confirm that the two WMH classes are uniquely associated with different cognitive abilities, imaging biomarkers and risk factors, suggesting that their distinction is clinically valid and that they may represent useful and distinct imaging biomarkers. However, longitudinal studies are needed to further explore the differential evolution overtime of the two WMH classes and their relevance to clinical variables and risk factors. In addition, since all classification criteria currently used in the literature are somewhat arbitrary (Barkhof and Scheltens, 2006; DeCarli et al., 2005; Sachdev and Wen, 2005), data-driven approaches should be used to explore whether other rules better separate the two classes according to specific clinical correlates of interest. For example, the distance from the ventricles that better separates the two classes could vary depending on the clinical variable of interest and be adapted according to brain size. Related to this, it should be noted that the dichotomization into PWMH and DWMH was first introduced in visual rating scales, to provide a more fine-grained quantification of the WMH load. The current increasing availability of automated segmentation algorithms for detailed mapping of WMH at the voxel-level will allow the study of the relationship with clinical variables in more detail, possibly replacing the current hard dichotomization with a continuous distribution (Barkhof and Scheltens, 2006; Griffanti et al., 2016; Rostrup et al., 2012).

In conclusion, our study suggests that the classification criterion used for the definition of PWMH and DWMH is not a major obstacle for the comparison of different studies. Among them, the 10 mm distance rule should give the most comparable results to the current literature in terms of volumes and showed the best separation between PWMH and DWMH in terms of association with the factors tested in this study. Our findings that PWMH and DWMH have different functional, microstructural and clinical correlates further support the hypothesis that they are indeed different entities and that their distinction can provide useful information about healthy and pathological aging processes.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.neuroimage.2017.03.024.

References

Allan, C.L., Sexton, C.E., Filippini, N., Topiwala, A., Mahmood, A., Zsoldos, E., Singh-Manoux, A., Shipley, M.J., Kivimaki, M., Mackay, C.E., Ebmeier, K.P., 2016. Sub-threshold depressive symptoms and brain structure: A magnetic resonance imaging study within the Whitehall II cohort. J. Affect. Disord. 204, 219–225.

Barkhof, F., Scheltens, P., 2006. Is the whole brain periventricular? J. Neurol. Neurosurg. Psychiatry 77, 143–144.

Bastin, M.E., Clayden, J.D., Pattie, A., Gerrish, I.F., Wardlaw, J.M., Deary, I.J., 2009. Diffusion tensor and magnetization transfer MRI measurements of periventricular white matter hyperintensities in old age. Neurobiol. Aging 30, 125–136.

Bolandzadeh, N., Davis, J.C., Tam, R., Handy, T.C., Liu-Ambrose, T., 2012. The association between cognitive function and white matter lesion location in older adults: a systematic review. BMC Neurol. 12, 126.

D'Agostino, R.B., Wolf, P.A., Belanger, A.J., Kannel, W.B., 1994. Stroke risk profile: adjustment for antihypertensive medication. The Framingham Study. Stroke 25, 40–43.

de Groot, J.C., de Leeuw, F.E., Oudkerk, M., Hofman, A., Jolles, J., Breteler, M.M., 2000. Cerebral white matter lesions and depressive symptoms in elderly adults. Arch. Gen. Psychiatry 57, 1071–1076.

De Groot, J.C., De Leeuw, F.E., Oudkerk, M., Van Gijn, J., Hofman, A., Jolles, J., Breteler, M.M., 2002. Periventricular cerebral white matter lesions predict rate of cognitive decline. Ann. Neurol. 52, 335–341.

Debette, S., Markus, H.S., 2010. The clinical importance of white matter hyperintensities on brain magnetic resonance imaging: systematic review and meta-analysis. BMJ 341, c3666.

DeCarli, C., Fletcher, E., Ramey, V., Harvey, D., Jagust, W.J., 2005. Anatomical mapping of white matter hyperintensities (WMH): exploring the relationships between periventricular WMH, deep WMH, and total WMH burden. Stroke 36, 50–55.

Fazekas, F., Chawluk, J.B., Alavi, A., Hurtig, H.I., Zimmerman, R.A., 1987. MR signal abnormalities at 1.5 T in Alzheimer's dementia and normal aging. AJR Am. J. Roentgenol. 149, 351–356.

Fazekas, F., Kleinert, R., Offenbacher, H., Schmidt, R., Kleinert, G., Payer, F., Radner, H., Lechner, H., 1993. Pathologic correlates of incidental MRI white matter signal hyperintensities. Neurology 43, 1683–1689.

Fernando, M.S., Simpson, J.E., Matthews, F., Brayne, C., Lewis, C.E., Barber, R., Kalaria, R.N., Forster, G., Esteves, F., Wharton, S.B., Shaw, P.J., O'Brien, J.T., Ince, P.G., Function, M.R.C.C., Ageing Neuropathology Study, G., 2006. White matter lesions in an unselected cohort of the elderly: molecular pathology suggests origin from chronic hypoperfusion injury. Stroke 37, 1391–1398.

Filippini, N., Zsoldos, E., Haapakoski, R., Sexton, C.E., Mahmood, A., Allan, C.L., Topiwala, A., Valkanova, V., Brunner, E.J., Shipley, M.J., Auerbach, E., Moeller, S., Ugurbil, K., Xu, J., Yacoub, E., Andersson, J., Bijsterbosch, J., Clare, S., Griffanti, L., Hess, A.T., Jenkinson, M., Miller, K.L., Salimi-Khorshidi, G., Sotiropoulos, S.N., Voets, N.L., Smith, S.M., Geddes, J.R., Singh-Manoux, A., Mackay, C.E., Kivimaki, M., Ebmeier, K.P., 2014. Study protocol: The Whitehall II imaging sub-study. BMC Psychiatry 14, 159.

Griffanti, L., Zamboni, G., Khan, A., Li, L., Bonifacio, G., Sundaresan, V., Schulz, U.G., Kuker, W., Battaglini, M., Rothwell, P.M., Jenkinson, M., 2016. BIANCA (Brain Intensity AbNormality Classification Algorithm): a new tool for automated segmentation of white matter hyperintensities. Neuroimage 141, 191–205.

Kim, K.W., MacFall, J.R., Payne, M.E., 2008. Classification of white matter lesions on magnetic resonance imaging in elderly persons. Biol. Psychiatry 64, 273–280.

Krishnan, M.S., O'Brien, J.T., Firbank, M.J., Pantoni, L., Carlucci, G., Erkinjuntti, T., Wallin, A., Wahlund, L.O., Scheltens, P., van Straaten, E.C., Inzitari, D., Group, L., 2006. Relationship between periventricular and deep white matter lesions and depressive symptoms in older people. The LADIS Study. Int. J. Geriatr. Psychiatry 21, 983–989.

Marmot, M., Brunner, E., 2005. Cohort Profile: the Whitehall II study. Int. J. Epidemiol. 34, 251–256.

Rostrup, E., Gouw, A.A., Vrenken, H., van Straaten, E.C., Ropele, S., Pantoni, L., Inzitari, D., Barkhof, F., Waldemar, G., group, Ls, 2012. The spatial distribution of age-related white matter changes as a function of vascular risk factors–results from the LADIS study. Neuroimage 60, 1597–1607.

Sachdev, P., Wen, W., 2005. Should we distinguish between periventricular and deep white matter hyperintensities? Stroke 36, 2342–2343, author reply 2343-2344.

Scheltens, P., Barkhof, F., Leys, D., Pruvo, J.P., Nauta, J.J., Vermersch, P., Steinling, M., Valk, J., 1993. A semiquantative rating scale for the assessment of signal hyperintensities on magnetic resonance imaging. J. Neurol. Sci. 114, 7–12.

Schmidt, R., Schmidt, H., Haybaeck, J., Loitfelder, M., Weis, S., Cavalieri, M., Seiler, S., Enzinger, C., Ropele, S., Erkinjuntti, T., Pantoni, L., Scheltens, P., Fazekas, F., Jellinger, K., 2011. Heterogeneity in age-related white matter changes. Acta Neuropathol. 122, 171–185.

Tudorascu, D.L., Rosano, C., Venkatraman, V.K., MacCloud, R.L., Harris, T., Yaffe, K., Newman, A.B., Aizenstein, H.J., 2014. Multimodal MRI markers support a model of small vessel ischemia for depressive symptoms in very old adults. Psychiatry Res. 224, 73–80.

van den Heuvel, D.M., ten Dam, V.H., de Craen, A.J., Admiraal-Behloul, F., Olofsen, H., Bollen, E.L., Jolles, J., Murray, H.M., Blauw, G.J., Westendorp, R.G., van Buchem, M.A., 2006. Increase in periventricular white matter hyperintensities parallels decline in mental processing speed in a non-demented elderly population. J. Neurol. Neurosurg. Psychiatry 77, 149–153.

Wardlaw, J.M., Smith, E.E., Biessels, G.J., Cordonnier, C., Fazekas, F., Frayne, R., Lindley, R.I., O'Brien, J.T., Barkhof, F., Benavente, O.R., Black, S.E., Brayne, C., Breteler, M., Chabriat, H., Decarli, C., de Leeuw, F.E., Doubal, F., Duering, M., Fox, N.C., Greenberg, S., Hachinski, V., Kilimann, I., Mok, V., Oostenbrugge, R., Pantoni, L., Speck, O., Stephan, B.C., Teipel, S., Viswanathan, A., Werring, D., Chen, C., Smith, C., van Buchem, M., Norrving, B., Gorelick, P.B., Dichgans, M., nEuroimaging, S.Tf. R.Vco, 2013. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. Lancet Neurol. 12, 822–838.

Wardlaw, J.M., Valdes Hernandez, M.C., Munoz-Maniega, S., 2015. What are white matter hyperintensities made of? Relevance to vascular cognitive impairment. J. Am. Heart Assoc. 4, 001140.

Wen, W., Sachdev, P., 2004. The topography of white matter hyperintensities on brain MRI in healthy 60- to 64-year-old individuals. Neuroimage 22, 144–154.

Wharton, S.B., Simpson, J.E., Brayne, C., Ince, P.G., 2015. Age-associated white matter lesions: the MRC Cognitive Function and Ageing Study. Brain Pathol. 25, 35–43.