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# Vitamin D and white matter abnormalities in older adults: a cross-sectional neuroimaging study

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## Keywords:

brain, leukoaraiosis, magnetic resonance imaging, older adults, vitamin D, white matter

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**Background and purpose:** Morphological brain changes related to hypovitaminosis D have been poorly studied. In particular, the age-related decrease in vitamin D concentrations may explain the onset of white matter abnormalities (WMA) in older adults. Our objectives were (i) to investigate whether there was an association between serum 25-hydroxyvitamin D (25OHD) concentration and the grade of WMA in older adults and (ii) to determine whether the location of WMA was associated with 25OHD concentration.

**Methods:** One hundred and thirty-three Caucasian older community-dwellers with no clinical hydrocephalus (mean  $71.6 \pm 5.6$  years; 43.6% female) received a blood test and a magnetic resonance imaging scan of the brain. The grades of total, periventricular and deep WMA were scored using semiquantitative visual rating scales from T2-weighted fluid-attenuated inversion recovery images. The association of WMA with as-measured and deseasonalized 25OHD concentrations was evaluated with the following covariates: age, gender, body mass index, use of anti-vascular drugs, number of comorbidities, impaired mobility, education level, Mini-Mental State Examination score, medial temporal lobe atrophy, serum concentrations of calcium, thyroid-stimulating hormone and vitamin B12, and estimated glomerular filtration rate.

**Results:** Both as-measured and deseasonalized serum 25OHD concentrations were found to be inversely associated with the grade of total WMA (adjusted  $\beta = -0.32$ ,  $P = 0.027$ ), specifically with periventricular WMA (adjusted  $\beta = -0.15$ ,  $P = 0.009$ ) but not with deep WMA (adjusted  $\beta = -0.12$ ,  $P = 0.090$ ). Similarly, participants with 25OHD concentration  $<75$  nM had on average a 33% higher grade of periventricular WMA than those with 25OHD  $\geq 75$  nM ( $P = 0.024$ ). No difference in average grade was found for deep WMA ( $P = 0.949$ ).

**Conclusions:** Lower serum 25OHD concentration was associated with higher grade of WMA, particularly periventricular WMA. These findings provide a scientific basis for vitamin D replacement trials.

## Introduction

Hypovitaminosis D is very common in older adults, with nearly one in two older adults having hypovitaminosis D [1,2]. Besides its classical function of bone metabolism regulation, vitamin D has been shown in the past decade to have multiple biological targets mediated by its nuclear hormone receptor, the vitamin D receptor, including brain neurons, astrocytes and microglia [1–7]. Consistently, experimentation has

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demonstrated that vitamin D is active in the brain and is able to reverse some age-related brain changes [8]. Several actions are described, including participation in neurophysiology through genetic regulation of neurotransmitters, neurotrophins and dendritic growth [4,5,9], but also neuroprotective effects based on antioxidant and anti-inflammatory properties [4,5,8,10]. It even seems that the brain parenchyma has greater vitality with adequate vitamin D impregnation and is better able to resist ischaemic stress [11].

Aging brain is characterized by a non-specific increase in white matter abnormalities (WMA), called leukoaraiosis [12–14]. WMA are easily visualized on magnetic resonance imaging (MRI) either in the deep white matter (D-WMA) or in contact with the lateral cerebral ventricles (periventricular WMA, P-WMA). The clinical relevance of both D-WMA and P-WMA is related to the disruption of cortico-subcortical white matter tracts that connect important cognitive regions of the brain [15], making them risk factors associated with cognitive decline [16].

Morphological brain changes related to low vitamin D status have been poorly studied [17]. Since epidemiological studies have reported that older adults with hypovitaminosis D have more frequent and more severe cognitive disturbances than those with normal levels [3,18], and based on the experimentally described neurosteroid properties of vitamin D, it was hypothesized that the age-related decrease in vitamin D levels could explain, at least in part, the WMA observed in older adults. The objectives of the present study were (i) to investigate whether there was an association between serum 25-hydroxyvitamin D (25OHD) concentration and the grade of WMA in older adults, and (ii) if so to determine whether the location of the WMA was associated with 25OHD concentration.

## Materials and methods

### Participants

Our study involved 133 older community-dwellers (mean age  $71.6 \pm 5.6$  years; 43.6% female; 100% Caucasian) followed in the Memory Clinic of the University Hospital of Angers, France, and recruited in the Gait and Alzheimer Interactions Tracking (GAIT) study from November 2009 to July 2011. The GAIT study is an observational cross-sectional study designed to examine gait in older community-dwellers reporting subjective memory complaint. The sampling and data collection procedures have been described elsewhere in detail [19]. In summary, subjective memory complaint was documented using the Subjective

Memory Complaints Questionnaire [20] and the main exclusion criteria were age below 60 years, Mini-Mental State Examination (MMSE) score  $<10$  [21], inability to walk independently, a history of stroke, history of any acute medical illness within the past 3 months, current delirium, severe depression, and inability to understand or answer the study questionnaires. For the present analysis, subjects were excluded when a diagnosis of clinical hydrocephalus was made based on the neurological evaluation (triad of gait instability, cognitive dysfunction and urinary incontinence) [22]. All included study participants received a full medical examination, consisting of structured questionnaires and standardized clinical examination, a cerebral MRI scan and a blood test.

### White matter abnormalities

#### Scan protocol

Imaging of the brain was performed with a 1.5 T MRI scanner (Magnetom Avanto, Siemens Medical Solutions, Erlangen, Germany) using a standard MRI protocol [22] including T1-weighted magnetization prepared rapid acquisition gradient echo axial images (acquisition matrix  $256 \times 256 \times 144$ , FOV  $240 \text{ mm} \times 240 \text{ mm} \times 187 \text{ mm}$ , TE/TR/TI = 4.07 ms/2170 ms/1100 ms), T2-weighted fluid-attenuated inversion recovery (FLAIR) axial images (acquisition matrix  $256 \times 192$ , FOV  $240 \text{ mm} \times 180 \text{ mm}$ , slice thickness 5 mm, slice gap 0.5 mm, 30 slices, TE/TR/TI = 122 ms/9000 ms/2500 ms), T2-weighted turbo spin-echo axial images, and T2\*-weighted axial images.

#### WMA measurements

The grade of WMA was evaluated from the T<sub>2</sub>-weighted FLAIR MR images, under the supervision of two neuroradiologists (TA and IBG), by a single observer (CA) who was blinded from participants' clinical information, including age, gender, prior imaging findings, comorbidities and cardiovascular risk factors, amongst others.

The total extent of white matter signal-intensity abnormality was measured using the semiquantitative visual rating scale devised by Manolio *et al.* [23], with a score ranging from 0 to 9 (worst). The inter-rater agreement of this scale is fair, with Cohen  $\kappa = 0.59$  [24]. D-WMA and P-WMA were characterized from the T<sub>2</sub>-weighted FLAIR images using the semiquantitative visual rating scale devised by Fazekas *et al.* [25]. D-WMA were scored on a four-point scale of increasing severity, according to the following: 0, normal; 1, punctuate foci; 2, beginning confluence of foci; 3, large confluent areas. P-WMA were scored as follows: 0, normal; 1, 'caps' or pencil-thin lining; 2,

smooth ‘halo’; 3, irregular periventricular hyperintensities extending into the deep white matter. The reliability of the Fazekas scale is excellent, with an intra-operator correlation coefficient of 0.85 [26] and inter-rater agreement Cohen  $\kappa = 0.78$  [24].

### Serum vitamin D assessment

Venous blood was collected from resting participants at the time of the brain imaging acquisition. Serum 25OHD concentration, an effective indicator of vitamin D status [1,2], was measured by radioimmunoassay (DiaSorin Inc., Stillwater, MN, USA) in nanomoles (to convert to ng/ml, divide by 2.496). With this method, there is no interference of lipids, which is often observed in other non-chromatographic assays of 25OHD. The intra- and inter-assay precision was 5.2% and 11.3% respectively. All measurements were performed locally at the University Hospital of Angers, France.

Deseasonalized 25OHD concentrations were calculated by regressing the measured 25OHD concentrations (in nM) on the periodic function:

$$y_t = \beta_0 + \beta_1 \sin\left(\frac{2\pi t}{365}\right) + \beta_2 \cos\left(\frac{2\pi t}{365}\right),$$

where  $y_t$  denotes measured serum 25OHD concentration,  $t$  denotes the day of the year the sample was collected and  $\beta_j$  ( $j = 0, 1, 2$ ) are estimated regression coefficients. Then the residuals were added to the seasonal average to create a deseasonalized vitamin D concentration for each individual [27,28]. This provides a way to adjust for the seasonal variation of 25OHD given that blood samples were collected throughout the year. ‘January 1’ deseasonalized values were arbitrarily chosen for analysis [28], although in a periodic function any date would be expected to be equally informative (and subject to the same limitations). Given the assumptions and limitations inherent in deseasonalized analysis, parallel analyses using as-measured 25OHD values were also performed.

### Covariates

The following clinical variables were included as potential confounders in the statistical models: age, gender, body mass index (BMI), use of anti-vascular drugs, number of comorbidities, impaired mobility, education level and MMSE score. The serum concentrations of calcium, thyroid-stimulating hormone and vitamin B12, estimated glomerular filtration rate, and medial temporal lobe (MTL) atrophy were also used as covariates in the analysis.

### Assessment of clinical covariates

Clinical covariates were obtained from a physical examination and a standardized comprehensive geriatric assessment. BMI was calculated as weight divided by height<sup>2</sup> (kg/m<sup>2</sup>). Evaluation of chronic diseases was based on self-report and health status questionnaires. Comorbidities were diseases lasting at least 3 months or running a course with minimal change, whatever their nature or site. The treatments usually taken were determined from the primary care physician’s prescriptions and by questioning the patient, whatever the dosage schedule or route of administration, and regardless of the date of commencement. Anti-vascular drugs were defined as anti-hypertensive (i.e. diuretics, beta-blockers, calcium antagonists, angiotensin conversion enzyme inhibitors, agonists of angiotensin II receptors or central antihypertensive agents), anti-diabetic (i.e. insulin or oral anti-diabetic agents), lipid-lowering (i.e. statins or fibrates) and anti-platelet drugs (i.e. aspirin, clopidogrel, ticlopidin or dipyridamole). Impaired mobility was defined by gait velocity slower than 100 cm/s at usual pace [29] using an electronic portable walkway with pressure sensor pads connected to a computer (GAITRite Gold walkway, 972 cm long, active electronic surface area 792 × 610 cm, with a total of 29 952 pressure sensors, scanning frequency 60 Hz, software version 3.8; CIR System, Havertown, PA, USA). Education level was self-reported with a structured standardized questionnaire. Participants who did graduate studies were considered to have a higher level of education compared with those who did not. Finally, cognitive status was assessed with the Folstein MMSE score [21].

### Assessment of biological covariates

Serum calcium, creatinine, thyroid-stimulating hormone and vitamin B12 were determined using automated standard laboratory methods at the University Hospital of Angers, France. Because of the high prevalence of hypoalbuminemia in older adults, serum concentrations of albumin and calcium were used to correct the calcium value: corrected calcium = uncorrected calcium (mM) +  $([40 - \text{albumin (g/l)}] \times 0.02)$ . Estimated glomerular filtration rate was estimated using the Cockcroft–Gault formula  $([(140 - \text{age}_{\text{years}}) \times \text{weight}_{\text{kg}} / \text{creatinine}_{\mu\text{M}}] \times 1.04$  for females and  $\times 1.25$  for males).

MTL atrophy was characterized from T1-weighted images using the qualitative five-point visual scale by Scheltens *et al.* [30]. The score ranges from 0 (no atrophy) to 4 (severe atrophy) based on the height of the hippocampal formation and the surrounding cerebrospinal fluid spaces. In the analysis, MTL atrophy was

defined as a score  $>2$  at least on one side (right or left) [30]. The scans were rated by a single blinded rater (CA) under the supervision of two neuroradiologists (TA and IBG) (intra-rater weighted Cohen  $\kappa = 0.68$ ) [31].

### Statistical analysis

First, the participants' characteristics were summarized using means  $\pm$  standard deviations or frequencies and percentages, as appropriate. Secondly, linear regressions were used to examine the association between serum 25OHD concentration (independent variable) and the grade of WMA (dependent variable), whilst adjusting for potential confounders. Separate analyses were performed to predict total WMA, D-WMA and P-WMA from deseasonalized and as-measured 25OHD concentrations. In order to improve the clinical significance of our results, an increase of 25 nM was used to define a unit of serum 25OHD change. Finally, D-WMA and P-WMA were compared between participants with serum 25OHD concentration  $<75$  nM and those with 25OHD  $\geq 75$  nM using a two-sided Student *t* test. *P* values  $<0.05$  were considered significant. All statistics were performed using SPSS (v20.0; IBM Corporation, Chicago, IL, USA) and Stata (v12.1; Stata Corporation, College Station, TX, USA).

### Standard protocol approvals, registrations and patient consents

Subjects participating in the study were included after having given their written informed consent for research. The study was conducted in accordance with the ethical standards set forth in the Helsinki Declaration (1983) and the protocol was approved by the University of Angers Ethical Review Committee (CPP Ouest II – 2009–12).

### Results

As indicated in Table 1, the mean as-measured concentration of serum 25OHD was  $58.6 \pm 27.6$  nM, and the mean deseasonalized value of 25OHD was  $54.0 \pm 27.3$  nM. Forty serum assays were performed in spring, 21 in summer, 50 in autumn and 22 in winter ( $P < 0.001$ ). Mean grade of total WMA was  $2.4 \pm 1.8$  on the Manolio scale (/9). Mean grade of D-WMA was  $1.3 \pm 0.7$  on the Fazekas scale (/3), and mean P-WMA was  $1.0 \pm 0.9$  (/3). Thirty-five participants (26.3%) presented with MTL atrophy.

Linear regression showed an inverse association of deseasonalized 25OHD concentration with the grade of

**Table 1** Summary of the participants' characteristics ( $n = 133$ )

Characteristics	Cohort ( $n = 133$ )	
	Summary value	95% CI
<b>Demographic measures</b>		
Age, years	$71.6 \pm 5.6$ [65–89]	70.7; 72.6
Female gender, $n$ (%)	58 (43.6)	35.2; 52.0
Body mass index, $\text{kg}/\text{m}^2$	$25.9 \pm 4.0$ [16.6–48.2]	25.2; 26.6
Regular use of anti-vascular drugs <sup>a</sup> , $n$ (%)	46 (34.6)	26.5; 42.7
Number of comorbidities <sup>b</sup>	$2.1 \pm 1.7$ [0–8]	1.8; 2.4
Impaired mobility <sup>c</sup> , $n$ (%)	54 (40.6)	32.4; 48.9
Higher education level <sup>d</sup> , $n$ (%)	36 (27.1)	19.6; 34.7
MMSE score, /30	$26.9 \pm 3.4$ [12–30]	26.3; 27.5
<b>Serum measures</b>		
25OHD, nM		
As-measured values	$58.6 \pm 27.6$ [13–189]	53.9; 63.3
Deseasonalized values	$54.0 \pm 27.3$ [13.3–182.8]	49.4; 58.6
Calcium <sup>e</sup> , mM	$2.4 \pm 0.1$ [2.1–2.7]	2.4; 2.4
Estimated glomerular filtration rate, ml/min	$63.8 \pm 30.1$ [21.3–343.8]	58.7; 68.9
TSH, mUI/l	$1.9 \pm 1.1$ [0.1–5.9]	1.7; 2.1
Vitamin B12, ng/l	$466.1 \pm 205.8$ [150–1714]	431.1; 501.1
<b>Brain mapping measures</b>		
Grade of white matter abnormalities		
Total WMA <sup>f</sup> , /9	$2.4 \pm 1.8$ [0–9]	2.1; 2.7
Periventricular WMA <sup>g</sup> , /3	$1.3 \pm 0.7$ [0–3]	1.2; 1.4
Deep WMA <sup>h</sup> , /3	$1.0 \pm 0.9$ [0–3]	0.9; 1.2
Medial temporal lobe atrophy <sup>i</sup> , $n$ (%)	35 (26.3)	18.8; 33.8

Summary values presented as mean  $\pm$  standard deviation [range] where applicable. CI, confidence interval; MMSE, Mini-Mental State Examination; 25OHD, 25-hydroxyvitamin D; TSH, thyroid-stimulating hormone; WMA, white matter abnormalities. <sup>a</sup>Antihypertensive, anti-diabetic, lipid-lowering or anti-platelet drugs; <sup>b</sup>diseases lasting at least 3 months or running a course with minimal change; <sup>c</sup>usual gait velocity slower than 100 cm/s; <sup>d</sup>graduate studies; <sup>e</sup>corrected value; <sup>f</sup>Manolio scale; <sup>g</sup>Fazekas scale for periventricular white matter abnormalities; <sup>h</sup>Fazekas scale for deep white matter abnormalities; <sup>i</sup>score on Scheltens scale  $>2$  at least on one side.

P-WMA (unadjusted  $\beta = -0.13$ ; 95% CI  $-0.24, -0.02$ ;  $P = 0.020$ ), but not with D-WMA (unadjusted  $\beta = -0.03$ ; 95% CI  $-0.17, -0.11$ ;  $P = 0.645$ ). After adjustment for potential confounders, serum 25OHD concentration was inversely associated with the grade of total WMA (adjusted  $\beta = -0.32$ ; 95% CI  $-0.60, -0.04$ ;  $P = 0.027$ ) and P-WMA (adjusted  $\beta = -0.15$ ; 95% CI  $-0.26, -0.04$ ;  $P = 0.009$ ), but not D-WMA (adjusted  $\beta = -0.12$ ; 95% CI  $-0.27, 0.02$ ;  $P = 0.090$ )



**Table 2** Multiple linear regressions showing the cross-sectional associations between the grades of total, periventricular and deep white matter abnormalities (dependent variables)<sup>a</sup> and serum 25-hydroxyvitamin D concentration (independent variable)<sup>b,c</sup> adjusted for participants' characteristics (*n* = 133)

	Grade of white matter abnormalities <sup>a</sup>								
	Total white matter abnormalities			Periventricular white matter abnormalities			Deep white matter abnormalities		
	Adjusted $\beta$	95% CI	<i>P</i>	Adjusted $\beta$	95% CI	<i>P</i>	Adjusted $\beta$	95% CI	<i>P</i>
Serum 25OHD concentration <sup>b,c</sup>	-0.32	-0.60; -0.04	<b>0.027</b>	-0.15	-0.26; -0.04	<b>0.009</b>	-0.12	-0.27; 0.02	0.090
Age	0.05	-0.03; 0.12	0.206	0.04	0.01; 0.07	<b>0.013</b>	0.03	-0.01; 0.07	0.086
Female gender	-0.13	-0.77; 0.50	0.676	-0.14	-0.39; 0.11	0.267	-0.09	-0.41; 0.23	0.580
Body mass index	0.06	-0.02; 0.15	0.144	0.04	0.01; 0.07	<b>0.027</b>	0.02	-0.03; 0.06	0.479
Use of anti-vascular drugs <sup>d</sup>	0.25	-0.42; 0.91	0.471	0.04	-0.22; 0.30	0.760	0.01	-0.32; 0.34	0.958
Number of comorbidities <sup>e</sup>	-0.12	-0.33; 0.09	0.267	-0.04	-0.12; 0.04	0.329	-0.02	-0.12; 0.09	0.736
Impaired mobility <sup>f</sup>	-0.61	-1.26; 0.04	0.067	-0.13	-0.38; 0.13	0.340	-0.22	-0.55; 0.11	0.195
Higher education level <sup>g</sup>	0.43	-0.29; 1.15	0.238	0.08	-0.21; 0.36	0.598	-0.01	-0.37; 0.35	0.964
MMSE score	-0.09	-0.20; 0.03	0.159	0.01	-0.04; 0.05	0.885	-0.01	-0.07; 0.05	0.703
Serum calcium concentration <sup>h</sup>	3.93	0.73; 7.12	<b>0.017</b>	1.00	-0.26; 2.27	0.119	1.64	0.02; 3.27	<b>0.047</b>
Estimated glomerular filtration rate	-0.01	-0.01; 0.01	0.607	-0.00	-0.01; 0.01	0.714	-0.00	-0.01; 0.00	0.768
Serum TSH concentration	-0.22	-0.48; 0.05	0.113	-0.01	-0.12; 0.09	0.821	-0.06	-0.20; 0.07	0.375
Serum vitamin B12 concentration	0.01	-0.01; 0.01	0.486	0.00	-0.01; 0.01	0.949	0.00	0.00; 0.01	0.340
Medial temporal lobe atrophy <sup>i</sup>	1.13	0.39; 1.87	<b>0.003</b>	0.41	0.11; 0.70	<b>0.007</b>	0.56	0.18; 0.93	<b>0.004</b>

$\beta$ , coefficient of regression corresponding to a change in the grade of white matter abnormalities; CI, confidence interval; 25OHD, 25-hydroxyvitamin D; MMSE, Mini-Mental State Examination; TSH, thyroid-stimulating hormone. <sup>a</sup>Three different regression models were used to predict consecutively the grade of total, periventricular and deep white matter abnormalities; <sup>b</sup>deseasonalized value; <sup>c</sup>change per 25 nM of serum 25-hydroxyvitamin D concentration; <sup>d</sup>antihypertensive, anti-diabetic, lipid-lowering or anti-platelet drugs; <sup>e</sup>diseases lasting at least 3 months or running a course with minimal change; <sup>f</sup>usual gait velocity slower than 100 cm/s; <sup>g</sup>graduate studies; <sup>h</sup>corrected value; <sup>i</sup>score on Scheltens scale >2 at least on one side. Bold values indicate  $\beta$  significant (i.e., *P*-value < 0.05).

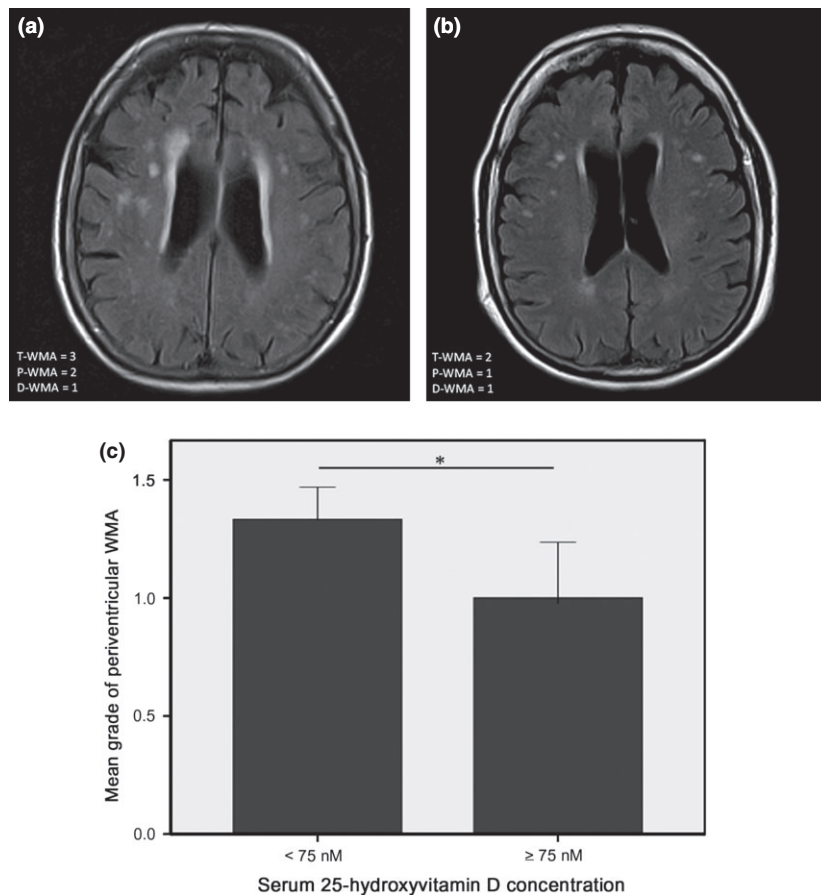
(Table 2). Using as-measured values of serum 25OHD concentration did not alter the associations with the grades of total WMA (adjusted  $\beta$  = -0.31; 95% CI -0.59, -0.03; *P* = 0.032) and P-WMA (adjusted  $\beta$  = -0.14; 95% CI -0.25, -0.03; *P* = 0.012). In addition, MTL atrophy was positively associated with the grade of total WMA, D-WMA and P-WMA. Increased calcium concentration was also associated with both the grade of total WMA and D-WMA (Table 2).

Finally, Fig. 1 shows representative examples of T<sub>2</sub>-weighted FLAIR MR axial images in participants with 25OHD < 75 nM and those with 25OHD  $\geq$  75 nM. The grade of P-WMA was higher in participants with 25OHD < 75 nM compared with the others, with a 33% difference (*P* = 0.024), but there was no difference for the grade of D-WMA (*P* = 0.949).

## Discussion

The main finding of this study performed in French older community-dwellers is that, irrespective of all measured potential confounders, serum vitamin D concentration was independently and inversely associated with total WMA in older adults. This association was found to involve specifically the P-WMA but not the D-WMA.

Despite accumulating evidence emphasizing the effects of vitamin D on the brain, particularly with regard to cognitive function [3,18], there are few studies on the impact of lower vitamin D levels on brain morphometry. To the best of our knowledge, only one observational study has explored the association of hypovitaminosis D with WMA thus far [32]. Those authors reported that older community-dwellers from the Boston area, MA, USA, had a larger volume and a more severe grade of WMA in the case of serum 25OHD < 25 nM than those with 25OHD > 50 nM. They also found that people with higher vitamin D dietary intakes  $\geq$ 400 IU/day had a smaller volume and less severe grade of WMA than the others [32]. However, this study did not consider the location of WMA, a crucial factor that may provide important information on the implicated mechanisms [12,13,33]. Moreover, the epidemiological approach was only descriptive in this first study, and no regression model was used to examine the association between serum 25OHD and WMA, which prevented the authors taking into account confounding factors such as calcium concentration. Yet, the link reported between hypovitaminosis D and WMA may have reflected covariation with this biological component related to both vitamin D status and vascular risk [34]. Despite these



**Figure 1** Representative examples of axial T2-weighted FLAIR magnetic resonance images in participants with insufficient (a) and normal (b) 25-hydroxyvitamin D status. (c) Bar plot representing the mean grade of periventricular white matter abnormalities for participants with vitamin D insufficiency and normal vitamin D status. Bar graphs indicate mean and 95% confidence interval. \* $P = 0.024$  for between-group comparison using the two-sided Student  $t$  test.

methodological divergences, both this previous study and the current study found that older adults with lower 25OHD status had a higher grade of WMA.

The inverse association between 25OHD status and WMA grade may have several possible explanations. First, older individuals with a high burden of WMA are more likely to have decreased cognitive and functional abilities and to be limited in their mobility [16] leading to lower intakes of vitamin D and thus to a greater risk of hypovitaminosis D. For instance, in multiple sclerosis, impaired mobility has been inversely correlated with reduced sun exposure and (subsequently) with lower serum 25OHD concentrations [35]. However, it is of note that our community-dwelling participants were relatively healthy with high mean BMI of  $25.9 \pm 4.0$  kg/m<sup>2</sup>, high mean MMSE score of  $26.9 \pm 3.4$  and high mean gait velocity of  $105.1 \pm 23.1$  cm/s (Table 1). Moreover, adjustment for these covariables did not alter the association of serum 25OHD concentration with WMA (Table 2), which makes this explanation less likely.

Alternatively, low vitamin D levels may have a role in precipitating WMA. The pathophysiology of WMA is not fully elucidated. D-WMA are generally attributed to cerebral ischaemic small vessel disease and hypertension, whilst P-WMA probably reflect age-related subcortical brain atrophy [12,13,33]. Interestingly, hypovitaminosis D may be involved in both of these pathological processes.

Vitamin D has demonstrated numerous links with vascular health. Specifically, a potential anti-atherosclerotic activity of vitamin D has been highlighted, and hypovitaminosis D has been associated with a higher prevalence of peripheral arterial disease [36]. Potential mechanisms for increased vascular disease risk include increased oxidative stress, lipid peroxidation, glucose intolerance and metabolic syndrome in the case of hypovitaminosis D [37,38]. Moreover, hypovitaminosis D appears to be a contributing factor to hypertension [39], mainly by suppression of the renin-angiotensin system expression in the juxtaglomerular apparatus [40]. Deleterious effects of



hypovitaminosis D on vascular brain health have already been described. A recent SPECT-CT (radio-nuclide brain Single-Photon Emission Computed Tomography/Computed Tomography) study in participants with Alzheimer's disease found that serum 25OHD concentration positively correlated with cerebral blood flow, those with hypovitaminosis D having decreased regional blood flow in the left precuneus cortex [41]. Consistent with this result, a meta-analysis showed that in both cross-sectional and longitudinal studies people with hypovitaminosis D have more strokes than others [42]. Furthermore, vitamin D appears not only to prevent cerebral vascular disorder but also to limit its consequences. For illustration, experiments in rats showed that vitamin D attenuated cortical infarction induced by cerebral arterial ligation [11]. Because ischaemic disease is a plausible determinant of WMA [12,13], increased brain sensitivity to vascular stress in the case of hypovitaminosis D may explain part of the hypovitaminosis-D-related WMA.

Since lower vitamin D status was associated in our study with P-WMA rather than D-WMA, our results suggest that the WMA observed in older adults with hypovitaminosis D may not be fully explained by cerebral ischaemic small vessel disease but rather by a loss of subcortical white matter. Experimental evidence precisely supports a role for vitamin D in regulating brain trophism and plasticity. Vitamin D appears to have a trophic function in the differentiation and maturation of neurons by controlling the rate of mitosis and the production and release of neurotrophins. Vitamin D promotes the synthesis of neurotrophic agents such as nerve growth factor, the glial-cell-line-derived neurotrophic factor and neurotrophin 3 [4,5,9]. It also accelerates dendritic growth in a dose-dependent manner in rodent hippocampal cell cultures [9]. *In vivo* observations in animals consistently find that vitamin-D-depleted rats present with enlarged lateral ventricles illustrative of subcortical atrophy [43]. In the same line, a recent study in humans confirmed that older adults with hypovitaminosis D had enlarged lateral ventricles (synonymous with subcortical parenchymal loss) compared with those with normal vitamin D status [22]. A previous report also found that a plasma nutrient pattern rich in vitamin D was associated with larger whole-brain volume on MRI [44]. These results strengthen the hypothesis that WMA observed in older adults with hypovitaminosis D in our study were probably due to subcortical atrophy.

The strengths of our study include the distinction of several locations of WMA (i.e. total WMA, P-WMA and D-WMA). Additionally, serum vitamin

D concentration was measured using a standardized and validated automated technique, and both as-measured and deseasonalized values of serum 25OHD concentrations were used in parallel analyses. Finally, regression models were applied to measure adjusted associations. Regardless, a number of limitations also exist. First, the study cohort was restricted to community-dwelling adults with memory complaint who might not be representative of all older adults. Secondly, although it was possible to control for important characteristics that could modify the association, residual potential confounders such as serum phosphorus concentration might still be present. Thirdly, the use of a cross-sectional design prevents any causal inference. Despite these limitations, it was possible to demonstrate a 33% increase in P-WMA associated with hypovitaminosis D. This new direction of research may offer a powerful mechanism to better understand the pathophysiology of cognitive disorders in older adults with lower vitamin D levels, and to act on their healthcare needs to maintain brain abilities late in life. Further prospective studies with different patient cohorts are warranted to clarify whether older adults with hypovitaminosis D are more likely to experience WMA and whether the correction of hypovitaminosis D could improve or prevent this process.

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The authors declare no financial or other conflicts of interest.

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