Joint effect of white matter lesions and hippocampal volumes on severity of cognitive decline. The 3C-Dijon MRI Study

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Running title: Brain MRI changes and rate of cognitive decline

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ABSTRACT

Background

Several brain MRI changes are observed in older individuals including White Matter Lesions (WML), Silent Brain Infarcts (SBI) and cerebral atrophy. Few studies have however assessed the combined association of these changes on the severity of future cognitive decline.

Methods

In the prospective population-based 3C-Dijon MRI study, 1701 non-demented participants aged 65 to 80 years at entry, had a brain MRI. Information on WML, Hippocampal volumes, SBI presence, and Brain Parenchymal Fraction were obtained. At 4-year follow-up, participants were screened for cognitive decline and dementia. Severity of cognitive decline was defined as none, moderate or severe calculated from neuropsychological test performance change. The relation between brain MRI markers and longitudinal change in cognition was studied using polytomous logistic regression and multiple linear regression models controlling for potential confounders. Two-by-two interactions were tested including with the ApoE genotype.

Results

At follow-up, 46 participants showed severe cognitive deterioration and 224 participants showed moderate cognitive deterioration. In multivariable analyses, risk of severe cognitive deterioration as well as the cognitive decline rate were significantly increased in participants with higher WML volume ($p<0.01$) and smaller hippocampal volume ($p<0.01$). The results suggested that WML and hippocampal volumes had a cumulative effect on the future level of cognitive decline. The APOE genotype was found to be an effect modifier of this association.

Interpretation

Vascular brain changes and degenerative processes coexist in normal older individuals. The co-occurrence of degenerative and non-degenerative pathologies could strongly affect the course of dementia expression.

KEYWORDS

Risk factors in Epidemiology; Cohort studies; Volumetric MRI; dementia; All cerebrovascular disease; Alzheimer's disease

Introduction

MRI defined vascular lesions (such as white matter lesions (WML) and silent brain infarcts (SBI)) and cerebral atrophy are highly prevalent in both demented and non demented older adults.[1-5] The impact of these age related brain modifications on cognitive function has naturally been investigated. WML and SBI have been associated with accelerated cognitive decline[6-12] and increased risk of dementia.[13-15] In the few population-based studies that have examined the cognitive consequences of global or regional cerebral atrophy the results suggest that both 'types' of atrophy are related to cognitive decline.[6, 10, 16, 17] Further findings from cross-sectional studies of a relationship between WML load and cortical atrophy[18, 19] suggest that several brain changes co-exist in older adults. How atrophy rate (global or regional) and vascular lesions interact to affect cognitive function has however not been elucidated. This is important as determining how, individually and in combination, these pathologies interact to cause cognitive deterioration and/or dementia risk. This is necessary to improve our understanding of the etiology of dementia and its prevention.

Previous studies have been hampered by small sample sizes of selected participants and the use of only one or two MRI brain markers simultaneously. The 3C-Dijon MRI study is a 4-year prospective community-based study of 1701 non-demented subjects aged 65 to 80 years old at baseline. Using this sample we assessed the association of several brain MRI markers on the rate of 4-year incident cognitive decline.

Material and methods

Sample

The 3C study is a multi-center, longitudinal population-based cohort study, conducted in three French cities (Bordeaux, Dijon, and Montpellier), and designed to estimate the risk of dementia and cognitive impairment attributable to vascular factors. A detailed description of the study protocol has been published elsewhere.[20] The study was approved by the Ethical Committee of the University Hospital of Kremlin-Bicêtre.

The current analyses were based only on data from the Dijon center where, between March 1999 and March 2001, 4931 non-institutionalized individuals aged 65 years or over, selected from electoral rolls, agreed to be enrolled in this research program. Each participant signed an informed consent and was followed-up at two-year intervals over 4 years.

A cerebral Magnetic Resonance Imaging (MRI) examination was proposed to those aged 65 to 80 years old who were enrolled between June 1999 and September 2000 (N=2763). Although 2285 subjects (82.7%) agreed to participate, because of financial limitations, 1924 examinations could be performed. Among subjects who had a MRI, 124 scans were excluded due to poor quality, a further 8 participants were excluded because they were demented at study entry. Subjects who had a valid MRI (N=1792) were on average younger (72.5 years (SD=4.1) versus 73.4 years (SD=4.0), $p<0.0001$), less often women (62.2% versus 71.0%, $p<.0001$), had an education level above baccalaureate (23.5% versus 17.8%, $p<0.0001$) and were overall in better health (data not shown) than subjects who refused MRI or whose MRI was not exploitable (N=971).

From the sample with a valid baseline MRI (N=1792), 1701 had at least one follow-up examination (1601 were followed-up to 4 years and 100 up to 2 years): 18 subjects died, 56 were lost to follow-up and 17 had no further cognitive assessment after inclusion. Subjects without follow-up data (N=91) were on average significantly older at entry (mean=73.2 years

vs. 72.3 years, p=0.04), had lower cognitive performance (mean Mini-Mental State Examination (MMSE[21]) score 27.0 versus 27.7, p<0.0005), and lower hippocampal volumes than subjects who remained in the study. No significant difference between the two groups was observed for gender, Brain Parenchymal Fraction (BPF) and WML volume.

Data collected

At each study wave, data were collected at the participants' home by a trained psychologist during a face-to-face interview using a standardized questionnaire. Information about demographic background, occupation, medical history, drug use and personal habits were collected. Each examination also included cognitive testing and diagnosis of dementia. Cognitive performances were assessed using a neuropsychological tests' battery which comprised the MMSE, Isaacs' Set Test (IST)[22], the Trail Making Test (TMT)[23], and the Benton Visual Retention Test (BVRT)[24]. Participants were screened for dementia using criteria based on the MMSE and IST scores corrected for education level, as previously validated in the PAQUID study.[25] For suspicious dementia cases, further information documenting cognitive disorders and ability in activities of daily living was obtained by the study centre clinician. Using DSM-IV criteria, the Clinical Dementia Rating scale as well as other information gathered during the clinical examination, the final diagnosis of dementia was made by an independent panel of five neurologists specialized in dementia.

For each neuropsychological test, annual cognitive decline was computed as the difference between the individuals' baseline score and their last available follow-up score divided by the delay between the two assessments (in years).

Cognitive deterioration was defined based on performance change in the MMSE, TMT part B, IST and BVRT. Three levels were defined including none, moderate and severe. Subjects were classified as having developed 'severe' cognitive deterioration between baseline and their last cognitive evaluation if they had been diagnosed as clinically demented (n=23) by the

panel of neurologists, or if their annual change in at least three tests was in the worst deciles (corresponding cut-offs were >0.50 for MMSE, >1.5 for IST, >0.75 for BVRT, <-1.90 for TMTB). Among the remaining participants, subjects were classified as having developed 'moderate' cognitive deterioration if their annual change in at least three tests was in the worst quartiles (corresponding cut-offs were >0.25 for MMSE, >0.75 for IST, >0.50 for BVRT, <-0.48 for TMTB), or if their annual change in two tests was in the worst deciles. [10, 26]

MRI data

MRI acquisition was performed on a 1.5 Tesla Magnetom (Siemens, Erlangen). A three-dimensional (3D) high-resolution T1-weighted brain volume was acquired using a 3D inversion recovery fast spoiled-gradient echo sequence (3D IR-SPGR; TR = 97 ms; TE = 4 ms; TI=600 ms; coronal acquisition). The axially reoriented 3D volume matrix size was 256 x 192 x 256 with a 1.0 x 0.98 x 0.98 mm^3 voxel size. T2- and PD- (proton density) weighted brain volumes were acquired using a 2D dual spin echo sequence with two echo times (TR = 4400 ms; TE1 = 16 ms; TE2 = 98 ms). T2 and PD acquisitions consisted of 35 axial slices 3.5 mm thick (0.5 mm between slices spacing), having a 256 x 256 matrix size, and a 0.98 x 0.98 mm^2 in-plane resolution.

Fully automatic image processing software was developed to detect, measure and localize WML.[27] Morphological parameters were computed for each detected WML including center of mass coordinates, Euclidian distance to the ventricular system and principal axis dimension. When its distance to the ventricular system was less than 10 mm, a WML was labeled as periventricular (PWML), otherwise it was labeled as deep (DWML). Stereotactic coordinates of the center of mass were calculated using the combination of the T1-to-PD/T2 and T1-to-Talairach space registration matrices. PWML and DWML volumes were calculated by summing the volumes of all the lesions detected in each area. The automatic method is 100% reproducible.

As WML volumes are estimated to be highly correlated to brain size, Total Intracranial Volume (TIV) was systematically adjusted for in all models. Total WML volume was studied as a continuous variable. Subjects were categorized as having high WML load (versus low) when total WML volume was in the fourth quartile of the sample distribution.

Presence of SBI was assessed visually by a neurologist (Y.Z.) using a standardized assessment grid. SBIs were defined as focal hyperintensities on T2-weighted images ≥ 3mm in size, with corresponding prominent hypointensities on T1-weighted images, with the same density as the cerebrospinal fluid.

Brain volumes estimation

The T1 and T2 weighted images for each subject were first aligned to each other[28] and then analysed with SPM99 (*http://www.fil.ion.ucl.ac.uk/spm/*). We used the optimized Voxel-Based Morphometry (VBM) protocol [29, 30] with two modifications to account for the structural characteristics of aged brains. First, grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF) templates specific to our database (3C-priors) were used for tissue segmentation. Second, segmentation of the CSF class was refined using T2 images.[31] The CSF partition images were derived from a multi-spectral combining T1 and T2 volumes, while the final GM and WM partition images were derived from the segmentation of the T1 volumes only. Finally, we applied a so-called "modulation" to each cerebral partition image, adjusting their voxel intensities for the strength of the deformation they were submitted to during the spatial normalization process. Modulation preserves the subject's original tissue quantity after its transfer to the reference space. For each subject, GM, WM, and CSF volumes were computed as the integral of the voxel intensities over the corresponding modulated tissue partition image. Total Intracranial Volume (TIV) was computed as the sum of the GM, WM and CSF volumes, and fractional cerebral compartment volumes as the ratios of tissue absolute volumes to TIV. We used brain parenchymal fraction (BPF), defined as the

ratio of brain tissue volume to total intracranial volume (BPF = (GM + WM)/TIV), as a marker of global brain atrophy. Volumes of gray matter in specific regions of interest (ROI) were computed by integrating within each of these ROIs, the voxel intensities of the modulated GM partition images. [32, 33] Anatomical limits were derived from a model of macroscopic neuroanatomical parcellation.[34] This parcellation is based on the high-resolution single-subject T1 volume provided by the Montreal Neurological Institute (MNI).[35] The gray matter map of the MNI for each subject was matched to that of the elderly population using a nonlinear spatial normalization. As gray matter images of all subjects were in the same stereotactic space, the same ROI limits were used to provide an estimate of the gray matter volume for each subject in each of the ROIs. For the purpose of this study, the ROI was the total hippocampal volume defined as the sum of the left and right hippocampal regions.[36] When hippocampal volume was below the 1st quartile (sex-specific), subjects were classified as having a small hippocampal volume. A similar rule was used to separate small and large BPF.

Covariates

Covariates included demographic and health variables collected at study entry. Education level was divided into four categories ranging from primary certificate level (low) to Baccalaureate or university degree (high). The presence of hypertension was defined as systolic blood pressure (SPB) ≥160 mm Hg, diastolic blood pressure (DBP) ≥ 95 mm Hg or the use of antihypertensive medication. Already-diagnosed diabetes, a fasting blood glucose ≥7 mmol/l or the use of diabetes medication was used to determine diabetes status. Hypercholesterolemia was defined by a cholesterol level ≥6.2mmol/L or by the use of lipid lowering drugs. Subjects were considered as having a history of cardiovascular disease if they reported a history of stroke, myocardial infarcts or arteritis. Apolipoprotein E genotype was studied in 2 classes: at least one e4 allele versus no e4 allele.

Statistical analysis

For each neuropsychological test, we used multiple linear regression models to assess the association between baseline brain MRI markers and annual rate of cognitive decline calculated as the difference between baseline and last assessments available divided by the delay. The relationship between baseline MRI brain markers and cognitive deterioration level (none, moderate, severe) was computed using multivariable polytomous logistic regression. Both linear and polytomous logistic regressions models were adjusted for potential confounders including age, sex, education level, Total Intracranial Volume (TIV), baseline cognitive performance, history of cardiovascular disease, hypertension, hypercholesterolemia, diabetes and Apolipoprotein E genotype.

To assess the joint association of WML volume and Hippocampus size on the risk of cognitive deterioration, we considered four groups of participants: Low WML + large Hippocampal (reference group), Low WML + small Hippocampal, high WML + large Hippocampal, High WML + small Hippocampal and we performed polytomous logistic regression adjusting for age, sex, education level, baseline MMSE score and TIV.

All two by two interactions were investigated by adding the cross-product term to the model and these analyses revealed a significant interaction between ApoE genotype, WML and hippocampal volume ($p=0.05$). Therefore, in a logistic regression model, we investigated how ApoE genotype could be an effect modifier on the association between high WML, small hippocampal volumes and risk of severe cognitive deterioration. For that purpose, subjects were classified into 8 groups corresponding to the combinations of E4 status (+ or -), hippocampal size (small or large), and WML load (low or high). The reference group was defined as E4-, large hippocampal and small WML volumes.

We finally performed a series of sensitivity analysis in order to assess the robustness of our findings. We first excluded subjects who had a stroke during follow-up (n=18). We also restricted the analyses to participants who had no silent brain infarcts on the brain MRI scan. We finally investigated the impact of lost to follow-up on our findings. For this purpose we used inverse probability weighting methods: we applied weights to the observed data such as if subject i had a probability of being followed-up of p_i, then this subject was given weight $1/p_i$ in the analysis.[37] Data analysis was completed using SAS (release 9.1; SAS Statistical Institute, Cary, NC).

Results

Table 1 shows the baseline demographics characteristics of the participants. Mean age was 72.3 years (Standard Deviation (SD) =4.1) and 60.7% of the sample was women. Mean baseline MMSE score was high (average=27.7, SD=1.7). Over the 4 years of follow-up, 46 participants showed severe cognitive deterioration and 224 participants showed moderate cognitive deterioration.

The association between brain MRI markers measured at baseline and annual decline in each neuropsychological test is shown in Table 2. Increased WML load was associated with greater decline on the MMSE, IST, and BVRT, but did not affect performance on the TMT B (p=0.07). Lower hippocampal volume was associated with a significantly higher rate of cognitive decline for all neuropsychological tests. Lower BPF was only associated with a greater MMSE decline and the presence of SBI at entry was only significantly associated with decrease performance on the TMT B.

Table 3 shows the relationship between each baseline brain MRI marker and severity of cognitive deterioration over 4-year follow-up. An increase of one SD in baseline WML volume was associated with a significant increased in the risk of severe cognitive deterioration (OR=1.2, 95% CI= (1.1-1.5)) after controlling for potential confounders. No association was observed between baseline WML and the risk of moderate cognitive deterioration (OR=1.0, 95% CI= (0.9 -1.2)). When WML volume was studied by location (periventricular or deep), the association with severe cognitive deterioration was slightly stronger for PWML but not statistically different from DWML. Subjects who had at least one SBI at MRI were not at increased risk of severe cognitive deterioration (OR=1.6, p=0.30). Higher baseline hippocampal volume was significantly associated with a decreased risk of future cognitive deterioration. The ‘protective’ influence of higher hippocampal volume was stronger on the risk of severe cognitive deterioration (OR=0.5, 95% CI= (0.3-0.7) per 1 SD

increase)) compared to moderate cognitive deterioration (OR=0.7 95% CI= (0.6-0.9) per 1 SD increase). BPF was not a significant predictor of cognitive deterioration severity.

When all 4 brain ageing markers were studied simultaneously in relation to 3-level cognitive outcome (Table 4), the relationships were similar to those found previously: both higher WML and smaller hippocampal volumes were independent predictors of severe cognitive deterioration, whereas neither BPF nor SBI predicted deterioration. Hippocampus size was significantly associated with moderate cognitive deterioration risk whereas WML severity, SBI presence and BPF were not.

We then investigated the combination of small hippocampal and large WML volumes in relation to cognitive deterioration risk (Table 5). Compared to subjects with both "normal" hippocampal and WML volumes (reference group), subjects with smaller hippocampal volume tended to have an increased risk of moderate cognitive deterioration whatever the WML load severity. Subjects with large WML load but "normal" hippocampal volume were not at increased risk of moderate cognitive deterioration. Compared to the reference group, subjects with small hippocampal volume only were at significantly increased risk of severe cognitive deterioration (OR=2.8, 95% CI= (1.2-6.5)). The risk of severe cognitive deterioration associated with higher WML volume only was of lower magnitude (OR=1.9, (95% CI= (0.7- 5.1)). However, subjects with both small hippocampal and high WML volumes had a higher risk of severe cognitive deterioration (OR=6.6, 95% CI= (2.7-16.2)). No such trend was observed for combined associations of other MRI markers on the risk of severe cognitive (results not shown).

Testing of two-by-two interactions revealed that ApoE genotype could be an effect modifier of the relationship between WML volume, hippocampal size and risk of severe cognitive deterioration (p=0.05) as shown in Figure 1. As compared with subjects with E4-, normal

hippocampal and WML volumes, subjects with both E4+ allele, smaller hippocampus and high WML load were at 36 times higher risk of severe cognitive deterioration (95% CI = 10 -132) whereas the risk of severe cognitive deterioration was 3.5 (95% CI=1.1- 11) in subjects with E4- allele, smaller hippocampus and high WML volumes. This pattern of results was consistently observed for the other participant groups (i.e., Low WML load + small hippocampal volume or High WML load + large hippocampal volume). The risk of severe cognitive deterioration associated with high WML load and small hippocampal volume (alone or combined) was increased in E4- but exacerbated in E4+ participants. Sex, age and education level were not found to be effect modifiers (results not shown).

Sensitivity analysis revealed that excluding subjects who had a stroke during follow-up (n=18) did not modify the results (not shown). Similarly, analyses restricted to subjects without SBI had little effect on the associations between brain ageing markers and cognitive deterioration severity (results not shown).

In addition, we also incorporated inverse weighting probability in our models in order to estimate the impact of differential drop-out on our findings. Between baseline and follow-up, 91 subjects dropped out. In the original model, the risk of severe cognitive deterioration associated with baseline WML volume was 1.2 (95% CI=(1.0-1.4)). In the weighted analysis, the corresponding risk was not modified (OR=1.2, 95% CI=(1.0-1.5)). Similarly, the estimated associations of hippocampus size, BPF or SBI presence at baseline with cognitive deterioration risk were unchanged between the non weighted and weighted analysis.

Discussion

In a large population-based sample of individuals aged 65 to 80 years old, we showed that higher WML volumes and a smaller hippocampus at baseline are independent predictors of future cognitive deterioration over 4-year follow-up. Our findings are also in favor of a cumulative effect of these two brain features on the risk of severe cognitive deterioration. Indeed, the risk of severe cognitive deterioration in subjects with both higher WML load and smaller hippocampal volume was more than double that of subjects having either higher WML load only or smaller hippocampal volume only. Our data also suggest that the APOE genotype might be an effect-modifier of the relationship between cerebral changes and cognitive deterioration, which is exacerbated in e4 carriers. The results did not suggest that BPF, global cerebral atrophy, or SBI are predictors of cognitive loss over 4-year follow-up.

Some limitations of our study should be considered. Compared with the general population of same age, the 3C-Dijon MRI study participants have higher education and socio-economic levels, and are overall healthier. Given this and the fact that MRI was proposed to participants aged 80 years and younger may explain the relatively low incidence of dementia in this study sample (1.4 per 1000 person-year). Further sample attrition may have biased the results. However, results from the weighted analysis suggests that dropout during follow-up did not impact the observed associations. In addition, the stability of the relationships even after controlling for numerous covariates reinforces the plausibility of the association observed. To study cognitive decline, slopes were calculated on two measurements. Analysis performed on the subsample of subjects who had three measurements available produced similar results (data not shown). We used an automated hippocampal volumetric approach which is not as accurate as manual or semi-automated measures but which is necessary when dealing with large samples size.

The strengths of our study include the sample size, the population based setting, the prospective design and the fully automated quantification of WML, BPF and hippocampal volume using methods which have been validated and are 100% reproducible.[38, 39]

The 3C-Dijon MRI study is the first large population-based study assessing the short term impact of WML load, SBI, brain and hippocampal volumes measured simultaneously, on future cognitive decline rate. Until now, population-based studies have investigated only one or two of the above brain ageing markers in relation to cognitive deterioration. In a sample of 224 non-demented subjects aged 65 years and older followed-up on average for six years, it was shown that higher WML volume was a predictor of mild cognitive impairment (MCI) whereas BPF was not.[40] In the European collaborative study CASCADE which included 1254 participants, cross-sectional analyses showed that periventricular WML and subcortical atrophy were associated with lower cognitive performances whereas cortical atrophy was not.[6] To our knowledge, only one prospective study has investigated concomitantly the consequences of vascular brain modifications, and global and regional atrophy on the risk of cognitive decline[16]. Here 120 subjects (some cognitively intact, others with MCI or dementia at baseline) were followed up over 3 years and it was found that cortical atrophy, but neither WML load or hippocampal atrophy were predictive of cognitive decline. However, the selected nature of the sample renders the interpretation of the findings limited.

Our results on WML are consistent with previous reports from population-based settings which also showed that higher WML load increases the risk of cognitive decline and dementia.[6, 8, 10, 12-14] Some of these studies have suggested that the relation between higher WML volumes and subsequent cognitive decline is limited to WML located in the periventricular area.[12, 13, 41] In the 3C-Dijon MRI sample, cognitive deterioration was more strongly associated with PWML volume than with DWML volume but the risks were not

statistically different. Several potential mechanisms may underlie the relation between WML and dementia. WML correspond to ischemic tissue damage, including infarction, gliosis, rarefaction and loss of myelin, that likely cause disconnection of functionally related cortical and sub-cortical structures important to cognitive function.[42-44] Rationale for a differentiation between PWML and DWML is debated. From pathophysiological observations it is suggested that the periventricular area has a high density of long associating fibers whereas the deep area has high density of short associating fibers. Therefore, the vascular architecture of periventricular area would be more vulnerable to WM damage and this could explain a differential impact on cognition.[45] There are also arguments against a distinction between PWML and DWML volumes. Indeed, analyses based on anatomical mapping of WML suggest that categorization between periventricular and deep WML is likely to be arbitrary.[46] Under this assumption, DWML would be contiguous to ventricular WML. Our findings support the latter hypothesis. Indeed, WML were categorized as periventricular if they were a distance of less than 10 mm to ventricular system. But changing the cutoff in sensitivity analyses did not influence the associations (results not shown).

Our data did not support the hypothesis that global brain fraction, a marker of global atrophy is a risk factor of future severe cognitive deterioration. We considered other markers such as CSF or grey matter volumes and the associations observed with cognitive outcome were similar to those reported with BPF (results not shown). There are few reports on cortical or regional atrophy in relation to cognitive deterioration in population-based samples, and where examined there is large heterogeneity in the definition of cortical atrophy used.[6, 17, 47, 48] In the Cardiovascular Health Study (CHS) which included 3608 subjects, severe cortical atrophy (visually rated from a 10-point scale) was a predictor of MCI over 5.8 years of follow-up.[47] In the Rotterdam study, both baseline hippocampal and amygdala volumes

(estimated using semi quantitative methods) were predictors of dementia onset over 6-years follow-up[17], but associations with global atrophy were not reported.

Reports from clinical settings studies are also conflicting. A report on 156 MCI patients suggested that BPF was a predictor of conversion to dementia[40], while in a sample of 129 MCI amnestic patients, hippocampal volume but not whole brain atrophy was predictive of progression to AD.[49]

Contrary to other reports, we do not show that SBI at study entry was significantly associated with cognitive decline rate.[15, 50] However, due to differences in definition, prevalence of SBI was lower in our study and the absence of significant relationship could reflect low power.

Whether the Apoe genotype mediates the consequences of MRI brain changes on dementia risk has only been previously investigated in one other study.[14] In the CHS study, subjects having both subcortical atrophy, high WML grade and carrying the Apoe e4 allele were 17 times more likely to develop dementia compared to subjects without any of these characteristics. However the above results as well as ours rely on small numbers and therefore, larger studies are needed to better understand the inter-relationship between Apoe genotype, brain changes at MRI and risk of dementia.

Large population-based studies, including ours, as well as neuropathological reports on large samples [51-53] demonstrate that degenerative processes (characterized by global or regional brain atrophy) and vascular changes co-occur in healthy individuals and could influence the course of clinical expression of dementia or cognitive deterioration. Quantitative assessment of vascular (WML load) as well as neurodegenerative (e.g., hippocampal volume) changes on brain MRI scans could be an important tool for identifying subjects at high risk of future dementia that could benefit from appropriate pharmacological interventions.[54]

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Disclosure Statement:

The authors report no actual or potential conflicts of interest in relation to this manuscript.

Table 1: General characteristics of the participants at study entry

	All sample	
	n	%
Female gender	1032	60.7
Mean age (SD)	72.3 (4.1)	
Education level		
Low	271	15.9
Medium low	752	44.2
Medium high	321	18.9
High	356	20.9
History of cardiovascular disease*	133	7.8
Hypertension†	1011	59.4
Diabetes‡	143	8.4
Hypercholesterolemia§	960	56.8
Drinking status n, %		
Current drinker	1364	80.5
Non drinker	301	17.8
Former drinker	30	1.8
Tobacco consumption n, %		
Non smoker	1054	62.0
Ex smoker	556	32.7
Current smoker	91	5.4
At least one ε4 carried for APOE genotype	365	21.6
Mean MMSE score (SD)**	27.7 (1.7)	
MRI markers		
Presence of Silent Brain Infarcts	170	10.0
Mean WML†† volume (SD), in cm^3	5.5 (4.9)	
Mean Hippocampal volume (SD), in mm^3	6.6 (0.8)	
Mean Brain Parenchymal Fraction (SD), in %	71.8 (3.1)	

* Self reported history of stroke, arterits and myocardial infarction
† systolic blood pressure>= 160 mm Hg and/or diastolic blood pressure >= 95 mm Hg or use antihypertensive medication
‡ Defined as reported diabetes or treatment for diabetes or glycemia >= 7 mmol/l
§ Treatment for hypercholesterolemia or cholesterol ≥ 6.2 mmol/L
** Mini-Mental State Examination
†† White Matter Lesion

Table 2: Association between MRI markers at baseline and annual change in cognitive performances

	NEUROPSYCHOLOGICAL TESTS							
	MMSE*		**Set Test Isaac**		**Test de Benton**		**TMT B†**	
	Beta (se)	P‡	Beta (se)	P	Beta (se)	P	Beta (se)	P
MRI markers								
WML§ volume	0.04 (0.01)	0.0003	0.21 (0.03)	<0.0001	0.03 (0.01)	0.04	-0.14 (0.08)	0.07
Hippocampal volume	-0.07 (0.01)	<0.0001	-0.17 (0.04)	<0.0001	-0.06 (0.02)	<0.0001	0.23 (0.10)	0.02
Brain Parenchymal Fraction	-0.04 (0.01)	0.003	-0.04 (0.04)	0.32	-0.01 (0.01)	0.36	0.06 (0.09)	0.54
Silent Brain Infarcts	-0.01 (0.04)	0.75	0.22 (0.07)	0.08	-0.002 (0.04)	0.96	-0.66 (0.28)	0.02

* Mini-Mental State Examination
† TMT: Trail Making test B
‡ Multiple linear regression models for 1 SD increased (except for SBI) adjusted for sex, age, education level, hypertension, history of cardiovascular disease, diabetes, MMSE score, hypercholesterolemia and ApoE genotype and TIV
§ WML: White Matter Lesions

Table 3: Separate analysis of each baseline Brain MRI marker and the risks of cognitive deterioration (no, moderate, severe) over 4-year follow-up

	LEVEL OF COGNITIVE DETERIORATION OVER 4-YEAR FOLLOW-UP				
	None N=1431	**Moderate N=224**		**Severe* N=46**	
		OR [95% CI] †	**P**	**OR [95% CI] †**	**P**
Total WML volume‡	1(ref)	1.0 [0.9 - 1.2]	0.53	1.2 [1.1-1.5]	0.02
WML volume by type ‡					
Periventricular WML	1(ref)	1.1 [0.9 - 1.3]	0.37	1.4 [1.0 – 2.1]	0.04
Deep WML	1(ref)	1.1 [0.9 - 1.3]	0.52	1.3 [0.9 - 1.9]	0.17
Hippocampal volume‡	1(ref)	0.7 [0.6 - 0.9]	0.0008	0.5 [0.3 - 0.7]	<0.0001
Brain Parenchymal Fraction‡	1(ref)	0.8 [0.7 – 1.0]	0.14	1.0 [0.7 - 1.4]	0.98
Silent Brain Infarcts	1(ref)	0.8 [0.5 - 1.3]	0.39	1.6 [0.7 – 3.8]	0.30

*Severe cognitive deterioration or dementia
†OR=Odds Ratio. Computed from polytomous logistic regression (reference group=Normal cognition at follow-up) adjusted for sex, age, education level, hypertension, history of cardiovascular disease, diabetes, MMSE score, hypercholesterolemia and ApoE genotype and TIV
‡ Associated risk per 1 SD increment
WML= White Matter Lesion

Table 4: Multivariable analysis of brain MRI markers at baseline and the risks of cognitive deterioration over 4-year follow-up

	LEVEL OF COGNITIVE DETERIORATION OVER 4-YEAR FOLLOW-UP		
	None N=1431	**Moderate N=224**	**Severe* N=46**
		OR† [95% CI]	**OR† [95% CI]**
Total WML volume ‡	1(ref)	1.0 [0.9 - 1.2]	1.2 [1.0-1.4]
Hippocampal volume ‡	1(ref)	0.7 [0.6 - 0.9]	0.4 [0.3 - 0.6]
Brain Parenchymal Fraction ‡	1(ref)	0.9 [0.7 - 1.1]	1.3 [0.9 - 1.9]
Silent Brain Infarcts	1(ref)	0.8 [0.5 - 1.3]	1.4 [0.6 – 3.3]

*Severe cognitive deterioration or dementia
† OR=Odds Ratio. Computed from polytomous logistic regression (reference group=Normal cognition at follow-up) adjusted for sex, age, education level, hypertension, history of cardiovascular disease, diabetes, MMSE score, hypercholesterolemia and ApoE genotype and TIV
‡ Associated risk per 1 SD increment
WML=White Matter Lesion

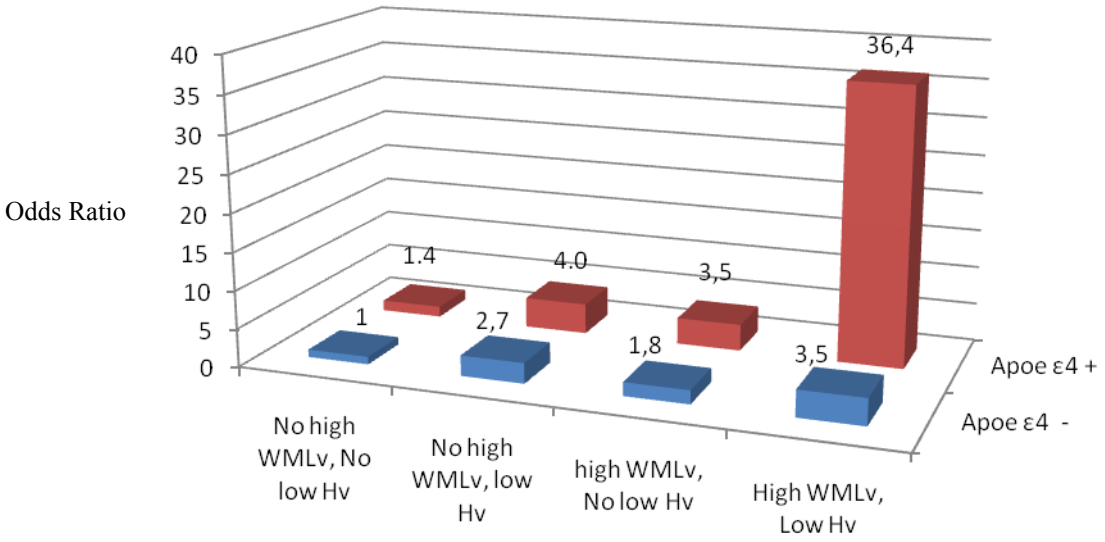
Table 5: Combined associations of small hippocampal and high white matter lesion (WML) volumes on the risks of cognitive deterioration

		LEVEL OF COGNITIVE DETERIORATION OVER 4-YEAR FOLLOW-UP					
		None (reference group)		Moderate		Severe	
		n		n	OR [95% CI]*	N	OR [95% CI] *
WML volume In percentile	**Hippocampal volume In percentile**						
$< 75^{th}$	$\geq 25^{th}$	864	1	111	1[ref]	12	1[ref]
$< 75^{th}$	$< 25^{th}$	229	1	54	1.4 [0.9 – 2.1]	15	2.8 [1.2 – 6.5]
$\geq 75^{th}$	$\geq 25^{th}$	256	1	35	1.0 [0.7 – 1.6]	7	1.9 [0.7 – 5.1]
$\geq 75^{th}$	$< 25^{th}$	82	1	24	1.8 [1.1 – 3.0]	12	6.6 [2.7 – 16.2]

* Odds Ratio adjusted for sex, age, education level, hypertension, history of cardiovascular disease, diabetes, hypercholesterolemia, ApoE genotype, MMSE score and TIV

75^{th} percentile WML=6.4 cm^3; 25^{th} percentile Hippocampal volume=6.0mm^3

Figure 1: Apoe4 genotype, small hippocampus, large White Matter Lesions (WML) volume and risk of severe cognitive deterioration over 4-year follow-up



WMLv: White Matter Lesion volume
Hv: Hippocampal volume
*Reference group= No high WMLv, No Low Hv

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