Original Article

JCBFM

Journal of Cerebral Blood Flow & Metabolism
0(00) 1–12

DOI: 10.1177/0271678X17707397
journals.sagepub.com/home/jcbfm
SAGE

Higher prevalence of cerebral white matter hyperintensities in homozygous *APOE-ε4* allele carriers aged 45–75: Results from the ALFA study

Santiago Rojas[1,2,*], Anna Brugulat-Serrat[1,*], Nuria Bargalló[3,4], Carolina Minguillón[1], Alan Tucholka[1], Carles Falcon[1,5], Andreia Carvalho[1,6], Sebastian Morán[7], Manel Esteller[7,8,9], Nina Gramunt[1], Karine Fauria[1], Jordi Camí[1,10], José L Molinuevo[1,11] and Juan D Gispert[1,5,10]

Abstract

Cerebral white matter hyperintensities are believed the consequence of small vessel disease and are associated with risk and progression of Alzheimer's disease. The *ε4* allele of the *APOE* gene is the major factor accountable for Alzheimer's disease heritability. However, the relationship between white matter hyperintensities and *APOE* genotype in healthy subjects remains controversial. We investigated the association between *APOE-ε4* and vascular risk factors with white matter hyperintensities, and explored their interactions, in a cohort of cognitively healthy adults (45–75 years). White matter hyperintensities were assessed with the Fazekas Scale from magnetic resonance images (575 participants: 74 *APOE-ε4* homozygotes, 220 heterozygotes and 281 noncarriers) and classified into normal (Fazekas < 2) and pathological (≥ 2). Stepwise logistic regression was used to study the association between pathological Fazekas and *APOE* genotype after correcting for cardiovascular and sociodemographic factors. *APOE-ε4* homozygotes, but not heterozygotes, bear a significantly higher risk (OR 3.432; 95% CI [1.297–9.082]; $p = 0.013$) of displaying pathological white matter hyperintensities. As expected, aging, hypertension and cardiovascular and dementia risk scales were also positively associated to pathological white matter hyperintensities, but these did not modulate the effect of *APOE-ε4/ε4*. In subjects at genetic risk of developing Alzheimer's disease, the control of modifiable risk factors of white matter hyperintensities is of particular relevance to reduce or delay dementia's onset.

Keywords

Alzheimer's disease, Apolipoprotein E, cerebrovascular, magnetic resonance imaging, risk factors, small vessel disease

Received 19 October 2016; Revised 16 March 2017; Accepted 21 March 2017

[1]Barcelonaβeta Brain Research Center, Pasqual Maragall Foundation, Barcelona, Spain
[2]Faculty of Medicine, Department of Morphological Sciences, Unit of Human Anatomy and Embryology, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain
[3]Magnetic Resonance Imaging Core Facility, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain
[4]Centre Mèdic Diagnòstic Alomar, Barcelona, Spain
[5]Centro de Investigación Biomédica en Red de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Zaragoza, Spain
[6]MRC Center for Developmental Neurobiology, King's College London, London, UK
[7]Epigenetics and Biology Program (PEBC), Bellvitge Biomedical Research Institute (IDIBELL), Barcelona, Spain
[8]Department of Physiological Sciences II, School of Medicine, University of Barcelona (UB), Barcelona, Spain
[9]Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain
[10]Universitat Pompeu Fabra, Barcelona, Spain
[11]Alzheimer's Disease and Other Cognitive Disorders Unit, Hospital Clínic, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain

*These authors contributed equally to this article

Corresponding authors:
Juan D Gispert, Barcelona βeta Brain Research Centre – Pasqual Maragall Foundation, C/ Wellington, 30. Barcelona E-08005, Spain.
Email: jdgispert@fpmaragall.org
José L Molinuevo, Barcelona beta Brain Research Centre – Pasqual Maragall Foundation, C/ Wellington, 30. Barcelona E-08005, Spain.
Email: jlmolinuevo@fpmaragall.org

Introduction

Small vessel cerebrovascular pathology constitutes one of the most common findings in neuroimaging studies carried out in late adulthood and elderly individuals.[1,2] The most common of these lesions can be observed through computed tomography or magnetic resonance imaging (MRI) as areas of increased signal in the white matter, commonly in periventricular locations, and are referred to as white matter hyperintensities (WMH). The Fazekas scale[3] rates WMH on a 0–3 point scale in the periventricular and subcortical regions combined. The Fazekas scale was designed for cross-sectional rating of WMHs, shows good intra- and inter-observer agreement and closely correlates with volumetric assessments.[4,5]

It is widely accepted that the etiology of these WMH is of ischemic origin, but the precise underlying pathology is not completely understood.[6] In this way, several alterations have been described in brain samples of deceased patients ranging from local blood brain barrier alterations with leakage of plasmatic proteins to major myelin destruction and axonal loss.[7–9] Both the prevalence and extension of WMH are strongly associated with aging and cardiovascular risk factors (CRF), particularly hypertension.[10] The reported prevalence of WMH is highly variable, ranging from below 10% to over 90% of a given study's participants. This variability can be accounted for by differences in the age of participants included in these cohorts and their comorbidities.[11,12] Additional factors could also contribute to these differences, such as ethnicity and lifestyles of the evaluated samples as well as technical differences in the MRI protocols.[13–15]

From a clinical perspective, severe affectation of white matter could lead to vascular dementia and the presence of these lesions may also contribute to the development of cognitive impairment in other causes of dementia.[16,17] For instance, it has been suggested that the presence of WMH could have an additive deleterious effect to amyloid pathology in Alzheimer's disease (AD), facilitating the progression of cognitive deterioration.[18,19] The contribution of midlife vascular risk factors in future brain function deterioration is further supported by risk scores for vascular disease like those from the Framingham study, which predict the development of cognitive deterioration.[20] Similarly, the CAIDE (Cardiovascular risk factors, Aging, and Incidence of DEmentia) risk score not only correlates with the extension of WMH, but also predicts the development of dementia after a follow-up of three decades.[21,22]

Although the etiology of sporadic AD remains elusive, the *Apolipoprotein E* gene (*APOE*) genotype is the major known factor accountable for the heritability of the disease.[23] In this regard, the presence of one *APOE-ε4* allele increases the risk of developing AD by a factor of 3 approximately, and homozygotes for this allele have nearly 14 times higher risk than *APOE-ε3/ε3* individuals. By contrast, the *APOE-ε2* allele seems to confer resistance towards developing the disease.[24] In addition to its role in sporadic AD, the *APOE-ε4* allele has also been associated with atherosclerosis, a risk factor for the development of cerebral vascular lesions and, eventually, vascular dementia.[25] In this regard, it has been proposed that the *APOE* genotype could be an independent risk factor for the development of WMH.[26] Thus, the association of the *APOE-ε4* allele with the development of AD could be explained, at least in part, by the major presence of WMH and other vascular lesions in these patients, which, in turn, could facilitate the onset of cognitive impairment and, ultimately, lead to dementia. In this way, it has been suggested that *APOE-ε4* carriers could be more vulnerable to environmental factors that facilitate cognitive deterioration including some vascular risk factors such as fat dietary intake.[21]

Several authors have described a positive association between the *APOE-ε4* allele and the prevalence of WMH.[27–30] However, these works frequently included patients with relevant comorbidities or advanced age that increased the prevalence of WMH and could mask the effect of the *APOE* genotype. Moreover, the number of subjects homozygous for the *APOE-ε4* allele recruited in previous studies was, in general, too low to confidently evaluate whether the number of alleles could have an additive effect on WMH in the same way that occurs with the development of AD: the largest of them only included 21 homozygotes from a total of 1779 recruited subjects.[28]

In the present work, we sought to investigate the association between the presence of the *APOE-ε4* allele with WMH in a cohort of cognitively healthy adults aged between 45 and 75 years. In addition, we aimed to recruit a high number of *APOE-ε4* homozygotes, with the aim of assessing whether the number of *APOE-ε4* alleles could have an additive effect on WMH load. Finally, we aimed to study the association of vascular risk factors and their potential interaction with the *APOE-ε4* allele.

Materials and methods

Participants

The ALFA (for ALzheimer and FAmilies) parent cohort, established by the Barcelonaβeta Brain Research Center (BBRC), consists of 2743 cognitively healthy participants, aged between 45 and 75. The ethnicity of participants is very homogeneous, and the vast majority of them are white Caucasians born in Spain

and have Spanish ancestry. The ALFA study protocol was approved by the Independent Ethics Committee *Parc de Salut Mar Barcelona* and registered at Clinicaltrials.gov (Identifier: NCT01835717). For a complete description of the ALFA parent cohort and inclusion and exclusion criteria, please refer to Molinuevo et al.[31] In short, participants of the present study had a Clinical Dementia Rating (CDR) equal to 0 and were cognitively normal as determined by a neuropsychological screening test battery that included the Mini-Mental State Examination, the Memory Impairment Screen, the Time Orientation of The Barcelona Test II and verbal semantic fluency. We excluded from the study those subjects with major comorbidities that could have an impact on vascular integrity. To assess whether the number of *APOE-ε4* alleles could have an additive effect on WMH, 608 ALFA parent cohort participants without contraindications to brain MRI were selected to participate in the present study according to their *APOE* genotype, preferentially including *APOE-ε4* allele carriers. The purpose of this strategy was to achieve a sample which permitted us to distinctly study the impact on WMH of *APOE-ε4* heterozygosity and homozygosity as compared to *APOE-ε4* allele non-carriers.

Research ethics and patient consent

The study was approved by the Ethics Committee of the "Parc de Salut Mar" (Barcelona, Spain; MRI/FBB2014v1.0) and conducted in accordance to the directives of the Spanish Law 14/2007, of 3 July, on Biomedical Research. All participants accepted the study procedures by signing an informed consent.

Cardiovascular and dementia risk factors

At ALFA baseline assessment, participants were enquired about their familiar and personal medical history and chronic medication use was recorded. Participants' weight, height, blood pressure, and waist and hip circumference were measured. Participants were considered as hypertensive if their measured systolic blood pressure was $\geq$160 mmHg and/or if they were on antihypertensive medication and/or if they self-reported to suffer current hypertension. Participants were classified as having diabetes or hypercholesterolemia if they were on anti-diabetic or anti-hypercholesterolemic medication, respectively. In addition, the presence of these comorbidities was also taken as positive if self-reported. Participants' weight and height measurements were used to calculate their body mass index (BMI). Smoking habits were collected using a simplified version of the 2009 Spanish Ministry of Health National Plan on Drugs Questionnaire. Finally, participants' level of physical activity was measured with the Spanish short version of the Minnesota Leisure Time Physical Activity Questionnaire[32] and they were categorized as active if exercised at least 150 min per week of moderate workout or 75 min per week of vigorous workout as recommended by the current guidelines.

Cardiovascular and dementia risk scores

Clinical and lifestyle-related variables gathered were used to estimate participants' risk of suffering coronary disease events at 10 years, using the REGICOR cardiovascular risk function (an adaptation of the Framingham function validated in a Spanish sample[33]), and the probability of dementia in 20 years, using the CAIDE dementia risk score, a well-established approach to assess the probability of dementia in late life according to the risk score categories in middle age.[34] Participants' risk scores were estimated using both CAIDE statistical models that differ in whether they do not include (model I) or include (model II) the *APOE* status into consideration.

The estimated variables were as follows: (i) *For the risk of suffering coronary disease events*. Total cholesterol: if a participant did not self-report to suffer hypercholesterolemia and they were not on anti-hypercholesterolemic medication, the assigned cholesterol value was $\geq$160 and $<$200 mg/dl, corresponding to a coefficient of 0 in the REGICOR Cox model. If they self-reported hypercholesterolemia and/or were on anti-hypercholesterolemic medication, the assigned value was $\geq$240 and $<$280 mg/dl, corresponding to a coefficient of 0.5054 and 0.2439 for men and women, respectively, in the same model. HDL-cholesterol: Participants were assigned a $\geq$45 to $<$50 mg/dl value, corresponding to a coefficient of 0 and 0.1979 for men and women, respectively, in the REGICOR Cox model. (ii) *For the probability of dementia in 20 years*. Total cholesterol: if a participant did not self-report to suffer hypercholesterolemia and they were not on anti-hypercholesterolemic medication, the assigned cholesterol value was 6.5 mmol/L, corresponding to a coefficient of 0 in the CAIDE Cox model. If they self-reported hypercholesterolemia and/or were on anti-hypercholesterolemic medication, the assigned value was 6.6 mmol/L, corresponding to a coefficient of 0.631 and 0.460 in model I and II, respectively.

Apolipoprotein E genotyping

Participants' *APOE* genotype was determined as described in Molinuevo et al.[31]

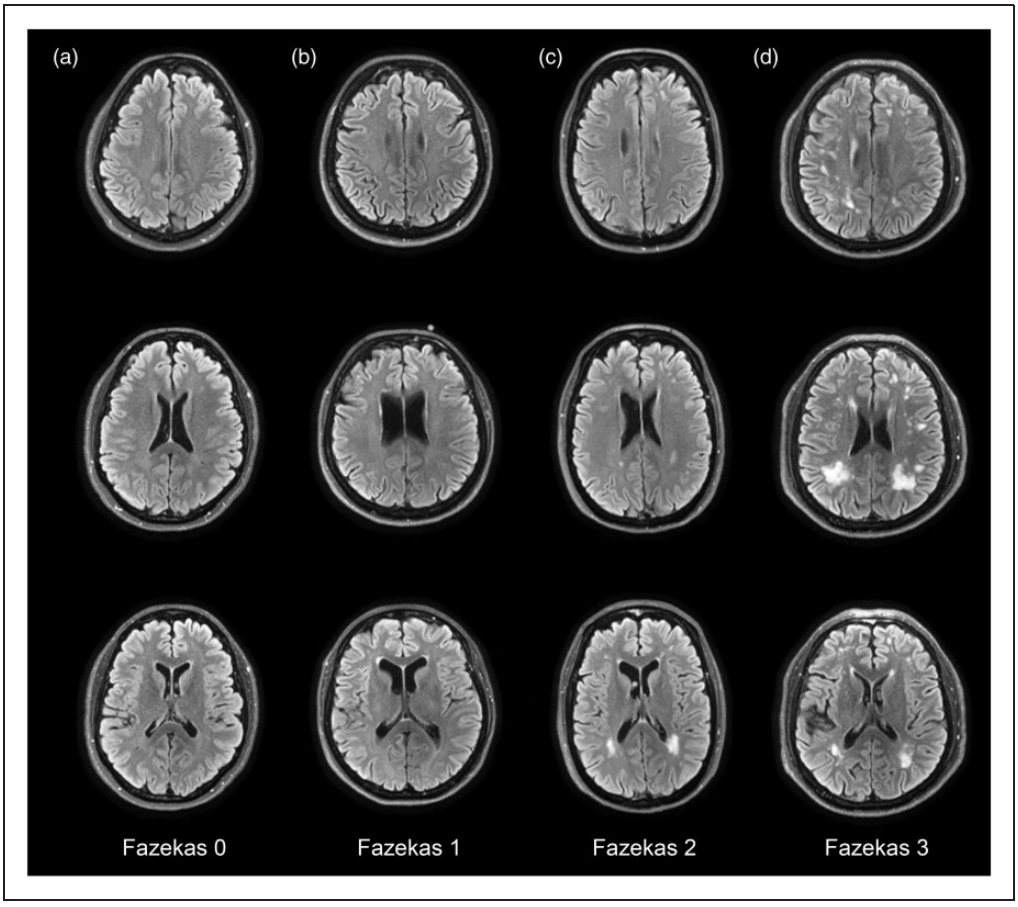

Figure 1. T2-FLAIR images illustrating the Fazekas Scores (A, 0; B, 1; C, 2 and D, 3).

MRI acquisition

Scans were acquired using a 3.0-T scanner (GE Discovery MR750 W 3T). The MRI protocol was identical for all subjects and included high-resolution T1-weighted 3D structural images with an isotropic voxel size of 1 mm^3 (TR/TE/TI = 8.0/3.7/450 ms, NSA = 1, *Flip angle* = 8° and a matrix size of $256 \times 256 \times 160$), as well as three T2-weighted pulse sequences (256×256, $1 \times 1 \times 3$ mm matrix): (i) Fluid attenuation inversion recovery (FLAIR: TR/TE/TI = 11000/90/2600 ms, *Flip angle* = 160°), (ii) fast spin echo (FSE: TR/TE = 5000/85 ms, *Flip angle* = 110°) and (iii) gradient-recalled echo (GRE: TR/TE = 1300/23 ms, *Flip angle* = 15°).

Radiological reporting of white matter hyperintensities

Scans were evaluated by a trained neuroradiologist within the following week after MRI acquisition and blind to the *APOE-ε4* status of the participants. The grades of WMH were evaluated using modifications of the Fazekas Scale,[3] which separately categorizes the severity of deep and periventricular lesions, on a scale from 0 to 3 (0: None or a single punctate WHM lesion, 1: Multiple punctate lesions, 2: Beginning confluency of lesions [bridging] and 3: Large confluent lesions) (Figure 1). For data analyses, the variable was dichotomized, classifying participants into two groups, namely Fazekas score <2 and score ≥2, the latter usually considered as pathological in individuals younger than 75 years old.[35–37]

Statistical analyses

An initial descriptive analysis of the study sample was made and participants were divided into three groups according to the number of *APOE-ε4* alleles of their *APOE* genotype. A between-group one-way ANOVA or a Kruskal–Wallis test (depending on the normality of the variables as assessed by a Kolmogorov-Smirnoff test; $p < 0.05$) was conducted to assess the effect of each of the variables.

A stepwise logistic regression analysis was conducted to predict the presence of a pathological Fazekas score (≥2). Participants' *APOE* status was codified in three different ways that are based on (i) Model 1, the *APOE*

haplotype (6 groups: "2/2", "2/3", "3/3", "2/4", "3/4", "4/4"), (ii) Model 2, the number of *APOE-ε4* alleles [3 groups: "0" (2/2, 2/3, 3/3); "1" (2/4, 3/4); "2" (4/4)] and (iii) Model 3, *APOE-ε4* carriers/non-carriers [2 groups: "Non-carriers" (2/2, 2/3, 3/3); "Carriers" (2/4, 3/4, 4/4)]. Aside from the *APOE-ε4* status, all models included the following predictors: hypertension, hypercholesterolemia, BMI and smoking habits, as well as sex, age and years of education as sociodemographic factors. A multinomial regression model to assess for the association of the Fazekas score as an ordinal variable was used (collapsing score = 3 with score = 2).

Regarding dementia risk estimates (CAIDE), a stepwise logistic regression analysis was also conducted, using the percentage of risk as a continuous variable. The analysis was performed for both CAIDE model I (model 4) and model II (model 5) and *APOE* status categorized as *APOE-ε4/4* homozygote or not. The predictors introduced in Models 1–3 were not included here since they are already considered in the CAIDE function.

Finally, Model 6 represents a stepwise logistic regression analysis in which the estimated 10-year cardiovascular percentage of risk was used as a continuous variable. *APOE* status was categorized as *APOE-ε4/4* homozygote or not. Education, the only predictor not included in the REGICOR function, was included in the analysis.

Initially, all predictors were introduced in the models along with all two-way interactions. Progressively, factors showing p values >0.1 were removed from the model except for sociodemographic factors. SPSS 17.0 for Windows was used for all the statistical analyses. Differences were considered to be statistically significant at $p < 0.05$ in all analyses.

Results

From the 608 ALFA parent cohort participants that were invited to take part in the present study, 575 provided valid MRI scans. The mean age was 57.79 years and 39.48% were men. Two hundred and eighty-one participants (48.9%) were *APOE-ε4* non carriers, out of whom 7 (1.2% of the entire MRI sample) were *APOE-ε2/2*, 111 (19.3%) were *APOE-ε2/3* and 163 (28.3%) were *APOE-ε3/3*. The group of *APOE-ε4* carriers was composed of 294 individuals (51.1%), out of whom 174 (30.3%) were *APOE-ε3/4*, 46 (8.0%) were *APOE-ε2/4* and 74 (12.9%) were *APOE-ε4/4* homozygous. The main demographic characteristics of the participants are shown in Table 1. There were significant differences in age (*APOE-ε4/4* individuals were younger than the rest), in the presence of hypercholesterolemia (the percentage of hypercholesterolemic participants increased with the number of *APOE-ε4* alleles) and in the percentage of risk of dementia estimated by the CAIDE model II (*APOE-ε4* allele carriers presenting a higher one). No significant between-group differences were found for the other variables. Out of the 575 subjects included in the study, 273 were rated with a Fazekas score of 0, 257 with 1, 43 with 2 and only 2 with a score of 3. Table 2 shows the distribution of Fazekas score according to number of *APOE-ε4* alleles.

Table 3 shows the results of the different binomial logistic regression models for the association of Fazekas score with *APOE-ε4*. When dichotomizing WMH as normal and pathological (Fazekas score <2 and ≥2, respectively) and coding *APOE* status by haplotype (Model 1), the results of the binomial logistic regression showed that only *APOE-ε4* homozygotes (but not any other haplotype) positively correlated with the presence of pathological WMH ($p = 0.011$) along with age ($p = 0.001$) and hypertension ($p = 0.007$). Similarly, when coding *APOE* status by the number of *APOE-ε4* alleles (Model 2), age ($p = 0.001$), hypertension ($p = 0.008$) and *APOE-ε4/4* genotype ($p = 0.008$) positively correlated with the presence of pathological WMH. The results of the multinomial regression model for the association of Fazekas as an ordinal variable coding *APOE* status by the number of *APOE-ε4* alleles (Supplementary Table 1) concurred with this analysis. On the other hand, when *APOE-ε4* status was coded according to the presence of, at least, one *APOE-ε4* allele (Model 3), no significant effect of *APOE-ε4* was detected ($p = 0.350$). For all these three models, interactions did not reach the stipulated statistical threshold ($p > 0.1$) and, hence, were removed from the model.

The results of logistic regression models for the association of Fazekas score with cardiovascular and dementia risk scores and *APOE- ε4* are shown in Table 4. As expected, homozygosity for the *APOE-ε4* allele was positively associated with the Fazekas score [CAIDE Model I ($p = 0.001$), CAIDE Model II ($p = 0.011$) and REGICOR ($p = 0.001$)]. Again, interactions did not reach statistical significance and were excluded from the models.

Discussion

In this work, we assessed the impact of the *APOE* genotype on the prevalence of pathological levels of WMH in a sample of cognitively healthy, late-middle-aged subjects and their potential interactions with vascular risk factors. To evaluate whether the number of *APOE-ε4* alleles could have an additive effect on WMH, participants in this study were selected according to their *APOE* genotype, preferentially including *APOE-ε4* allele carriers, among members of the ALFA parent cohort. This strategy resulted in a highly enriched

Table 1. Characteristics of participants according to number of *APOE-ε4* alleles.

		Number of *APOE-ε4* alleles			
	Total ($n=575$)	None ($n=281$)	One ($n=220$)	Two ($n=74$)	p
Age (years), Mean (SD)	57.79 (7.4)	58.2 (7.6)	58.3 (7.3)	54.7 (6.2)	0.001
Male sex, *n* (%)	227 (39.5)	102 (36.6)	98 (44.5)	27 (36.5)	0.148
Education (years), Mean (SD)	13.63 (3.5)	13.6 (3.6)	13.8 (3.5)	13.4 (3.5)	0.742
Hypertension[a], *n* (%)	151 (26.2)	75 (26.7)	61 (27.7)	15 (20.2)	0.440
Diabetes[b], *n* (%)	28 (4.9)	15 (5.3)	10 (4.5)	3 (4.1)	0.865
Cholesterol[b], *n* (%)	177 (30.8)	74 (26.3)	73 (33.2)	30 (40.5)	0.039
BMI, Mean (SD)	26.88 (4.1)	26.9 (3.9)	26.9 (4.3)	26.8 (4.3)	0.985
Underweight (<18.5), *n* (%)	1 (0.2)	0	1 (0.45)	0	
Normal weight (18.5–24.9), *n* (%)	194 (33.7)	93 (33.1)	78 (35.4)	23 (31.1)	
Overweight (25–29.9), *n* (%)	261 (45.4)	128 (45.5)	92 (41.8)	41 (55.4)	
Obesity (≥30), *n* (%)	119 (20.7)	60 (21.3)	49 (22.3)	10 (13.5)	
Smoking habits, *n* (%)					0.118
Non-smoker	92	42 (14.9)	40 (18.2)	10 (13.5)	
Ex-smoker	317	161 (57.3)	118 (53.6)	38 (51.3)	
Smoker	135	68 (24.2)	44 (20.0)	23 (31.1)	
Not available	31	10 (3.5)	18 (8.2)	3 (4.1)	
CAIDE score model I, *n* (%)					
0–5	234 (40.7)	122 (43.4)	82 (37.3)	30 (40.5)	
6–7	189 (32.9)	89 (31.7)	71 (32.3)	29 (39.2)	
8–9	100 (17.4)	44 (15.6)	44 (20.0)	12 (16.2)	
10–11	39 (6.8)	19 (6.6)	17 (7.7)	3 (4.1)	
≥12	4 (0.69)	3 (1.2)	1 (0.4)	0	
Not available	9 (1.6)	4 (1.5)	5 (2.3)	0	
Risk (%), Mean (SD)	2.2 (3.2)	2.2 (2.4)	2.4 (2.4)	1.5 (1.5)	0.192
CAIDE score model II, *n* (%)					
0–5	127 (22.1)	96 (34.2)	19 (8.6)	12 (16.2)	
6–7	142 (24.7)	79 (28.1)	45 (20.4)	18 (24.3)	
8–9	170 (29.6)	72 (25.6)	69 (31.4)	29 (39.2)	
10–11	84 (14.6)	23 (8.2)	51 (23.4)	10 (13.5)	
≥12	43 (7.5)	7 (2.5)	31 (14.1)	5 (6.7)	
Not available	9 (1.6)	4 (1.4)	5 (2.3)	0	
Risk (%), Mean (SD)	2.6 (3.2)	1.7 (2.0)	3.9 (4.1)	2.6 (2.3)	<0.001
REGICOR, *n* (%)					
Low	424 (73.8)	213 (75.8)	161 (73.2)	50 (67.6)	
Moderate	120 (20.8)	53 (18.9)	45 (20.4)	22 (29.7)	
High	17 (2.9)	9 (3.2)	7 (3.2)	1 (1.35)	
Very High	4 (0.8)	2 (0.7)	2 (0.9)	0	
Not available	10 (1.7)	4 (1.4)	5 (2.27)	1 (1.35)	
CV risk (%), Mean (SD)	4.3 (2.9)	4.2 (2.9)	4.5 (2.9)	4.3 (2.9)	0.428

BMI: body mass index; CV: cardiovascular. [a]Systolic blood pressure ≥160 mm Hg and/or use antihypertensive medication and/or self-reported. [b]Medications and/or self-reported.

sample for the *APOE-ε4/4* genotype which allowed us to distinctly study the impact on WMH of *APOE-ε4* heterozygosity and homozygosity as compared to non-carriers. Our main finding is that cognitively healthy late-middle-aged *APOE-ε4* homozygous bear a significantly higher risk (OR 3.591; 95% Confidence Interval [1.347–9.578]; $p=0.011$) of displaying pathological levels of WMH than non-carriers. On the other hand,

Table 2. Distribution of Fazekas Scale score according to number of *APOE-ε4* alleles.

	Number of *APOE-ε4* alleles		
	None ($n=281$)	One ($n=220$)	Two ($n=74$)
Fazekas Scale, *n* (%)			
Score 0: None or a singlepunctate lesion	127 (45.2)	109 (49.5)	37 (50.0)
Score 1: Multiple punctate lesions	134 (47.7)	96 (43.6)	27 (36.5)
Score 2: Beginning confluency of lesions	18 (6.4)	15 (6.8)	10 (13.5)
Score 3: Large confluent lesions	2 (0.7)	0 (0.0)	0 (0.0)

the risk of *APOE-ε4* heterozygous participants is not significantly different from that of non-carriers. As expected, age and hypertension were also positively associated to a pathological degree of WMH. Similarly, the observed association between presence of WMH with cardiovascular and dementia risk score estimates was also expected, in view of the existing literature.[22,38,39] Interestingly, none of the CRF analyzed here (namely diabetes, hypertension, hypercholesterolemia, BMI and smoking habits) showed an interaction with *APOE* status, indicating that the effect of *APOE* on WMH is independent of these factors.

Several studies have evaluated the association between *APOE* genotype and the prevalence and severity of WMH. In fact, some articles could not find any differences in WMH load between *APOE-ε4* allele carriers and non-carriers,[40,41] but others have observed a clear correlation.[29,42] These recruited subjects from general population, patients with cerebrovascular disease[43] or patients affected of probable AD.[44] There is, therefore, a great variability on the characteristics of subjects evaluated among studies, including age, ethnicity, comorbidities and other demographic factors that could have an impact on WMH. Consequently, it is not surprising that previous published works have reported contradictory results regarding the impact of the *APOE* genotype on WMH prevalence and severity. In general, those suggesting a possible role of the *APOE-ε4* isoform in WMH studied large cohorts recruited from the general population.[30] Recently, the results of a meta-analysis that addressed this topic have also reinforced the idea that, at least in general population, *APOE-ε4* allele carriers presented a higher risk of developing WMH.[26] By contrast, several studies that did not find any correlations were carried out in cohorts of patients affected from cerebrovascular disease or AD. Under these conditions, other factors that participate in the development of WMH, such as cardiovascular disease, could prevail over the effect of the *APOE* genotype and contribute to the observed lack of association. In our study, subjects with relevant medical pathology or neurologic disease were excluded. In consequence, our sample is healthier than could be expected from an age-matched cohort selected from the general population. Moreover, participants are younger in comparison with those in previous studies. Since comorbidities and age are risk factors to develop WMH, our selection criteria produced a cohort with a lower WMH load than others reported in the literature. The relatively low level of WMH pathology in our cohort might account for the lack of correlation in heterozygosis in our results. It could be speculated that an increased risk may be found in older *APOE-ε4* heterozygotes.

Another important consideration to take into account is the difference in the ethnicity of the subjects between studies. The association between carrying *APOE-ε4* alleles and WMH has been clearly observed in white Caucasians, thus it cannot be a priori generalized to other populations.[27] In this way, other studies carried out in Japanese patients did not observe a correlation between *APOE-ε4* and WMH.[40,45]

Aside of the demographic differences between the studied cohorts, the low number of *APOE-ε4/4* homozygotes undoubtedly represents the most relevant limitation in previous studies exploring the relationship between *APOE-ε4* status and WMH. Very few studies have analyzed the correlation between WMH and *APOE-ε4/4* homozygosity and the largest of them only included 21 homozygotes from a total of 1779 recruited subjects. In this work, *APOE-ε4/4* homozygotes showed more WMH and an increased progression of the lesions during a five-year follow up period. In the same way, it was also found that WMH load of *APOE-ε4/4* individuals is higher than that of their *APOE-ε3/4* counterparts.[28] A recent report on more than 2000 participants aged between 20 and 90 years did not discover any association with the *APOE* genotype, purportedly because they were not able to analyse homozygotes separately (the number of homozygotes is not reported in the paper).[41] Therefore, the major strength of our study is the number of recruited *APOE-ε4/4* homozygous ($n=74$) which is, to our knowledge, considerably higher than any other single center study published to date. This enabled us to characterize the association between *APOE-ε4* genetic load and WMH. In fact, no correlation with pathological WMH could be detected in our sample in association with *APOE-ε4* when we pooled all subjects presenting at least one *ε4* allele together. Nevertheless, it is worth mentioning that our sampling strategy was designed to strengthen the

Table 3. Logistic regression models for the association of Fazekas score with *APOE-ε4*.

		B	Odds ratio (95% CI)	*p*
MODEL 1				
Age (years)		0.083	1.087 (1.034–1.143)	0.001*
Education (years)		−0.018	0.982 (0.896–1.076)	0.693
Sex (reference: men)		−0.027	0.973 (0.501–1.890)	0.936
Hypertension		0.9198	2.506 (1.283–4.896)	0.007*
APOE-ε4 haplotypes	*n*			0.098
(reference: ε3/3)	163			
ε2/2[a]	7	−18.233	–	0.999
ε2/3	111	0.245	1.277 (0.461–3.537)	0.638
ε2/4	46	0.619	1.856 0.535–6.447)	0.330
ε3/4	174	−0.110	0.896 (0.377–2.126)	0.803
ε4/4	74	1.279	3.591 (1.347–9.578)	0.011*
$X^2 = 32.227$, df = 9, $p < 0.001$*				
MODEL 2				
Age (years)		0.079	1.082 (1.032–1.136)	0.001*
Education (years)		−0.021	0.979 (0.894–1.072)	0.645
Sex (reference: men)		−0.013	0.987 (0.509–1.915)	0.970
Hypertension		0.904	2.470 (1.267–4.814)	0.008*
Number of *APOE-ε4* alleles	*n*			0.015*
(reference: None)	281			
One *APOE-ε4*	220	−0.022	0.978 (0.479–1.997)	0.951
Two *APOE-ε4*	74	1.191	3.289 (1.371–7.893)	0.008*
$X^2 = 30.045$, df = 6, $p < 0.001$*				
MODEL 3				
Age (years)		0.067	1.069(1.021–1.119)	0.004*
Education (years)		−0.023	0.977 (0.893–1.069)	0.608
Sex (reference: men)		−0.020	0.980 (0.511–1.882)	0.953
Hypertension		0.883	2.418 (1.251–4.675)	0.009*
APOE-ε4	*n*			
(reference: non Carriers)	281			
Carriers	294	0.301	1.352(0.718–2.543)	0.350
$X^2 = 23.579$, df = 5, $p < 0.001$*				

[a]Was excluded due to small *n*. *$p \leq 0.005$.

ability to detect associations for *APOE-ε4* homozygotes. The limitation of this strategy is that the allele frequencies in our study are not representative of an unselected population, thus increasing the risk of bias and preventing us from drawing any epidemiological conclusions. In addition, even though the vast majority of the study participants are white Caucasians and, consequently, ethnically homogeneous, specific genotyping of ancestry informative markers in our study population has not been performed. Given that *APOE* alleles are highly stratified by ethnicity[46] even within white European populations, unmeasured social, cultural or genetic factors that vary by ethnicity and increase WMH could confound the observed associations.

On the other hand, a few studies have suggested an association between the *APOE-ε2* allele and increased levels of WMH as well as with other vascular cerebral alterations.[47] Our observations do not support this hypothesis since no correlation was found between WHM and this allele. However, other vascular lesions such as microbleeds or lacunar infarcts were too scarce in our cohort to allow us to perform correlational analyses.

Table 4. Logistic regression models for the association of Fazekas score with cardiovascular and dementia risk scores and *APOE-ε4*.

		B	Odds ratio (95% CI)	*p*
MODEL 4				
CAIDE Model I[a]		0.176	1.193 (1.079–1.319)	0.001*
APOE-ε4	*n*			
(reference: Others than *APOE-ε4/4*)	492			
APOE-ε4/4	74	0.936	2.549 (1.178–5.514)	0.017*
$X^2 = 13.979$, df = 2, $p = 0.001$*				
MODEL 5				
CAIDE Model II[a]		0.096	1.101 (1.022–1.186)	0.011*
APOE-ε4	*n*			
(reference: Others than *APOE-ε4/4*)	492			
APOE-ε4/4	74	0.819	2.269 (1.061–4.853)	0.035*
$X^2 = 9.092$, df = 2, $p = 0.011$*				
MODEL 6				
REGICOR[a]		0.141	1.151 (1.063–1.246)	0.001*
Education		−0.027	0.973 (0.889–1.066)	0.557
APOE-ε4	*n*			
(reference: Others than *APOE-ε4/4*)	492			
APOE-ε4/4	73	0.750	2.117 (0.958–4.676)	0.064
$X^2 = 14.634$, df = 3, $p = 0.002$*				

[a]Percentage of cardiovascular risk. *$p \leq 0.005$.

The increased WMH observed in *APOE-ε4/4* individuals could be related to an increased tendency towards vascular pathology, a higher sensibility to risk factors, a reduced resilience to brain injury and a reduced recovery of lesions.[48] In any case, the accumulation of WMH could decrease the efficiency of subcortical white matter to perform its function and may have additive or even synergistic effects with other noxious processes acting in the brain like neurodegeneration in the cortical grey matter.[16] In the last instance, accumulation of WMH could facilitate cognitive deterioration and the onset of AD dementia.[49,50] Hence, controlling WMH load in AD susceptible individuals could be a useful preventive strategy to reduce or delay cognitive decline.[51] In contrast with age or *APOE* genotype, other factors associated with WMH, like hypertension, are modifiable with changes in life style or pharmacological treatment. There is no doubt that the control of vascular risk factors is the way to go for all patients not only for these with increased risk of developing WMH. However, it could be theorized that a more strict control of these factors could particularly benefit the APOE-*ε4/4* homozygotes. The same rationale could be considered for the development of treatments focused on reducing WMH load and progression. Such interventions could reduce the likelihood of cognitive deterioration and eventually the onset of dementia in *APOE-ε4/4* homozygotes, which are at a higher risk of developing AD.[28]

Cognitively healthy *APOE-ε4* homozygotes have also been reported to show a significantly higher prevalence of cerebral amyloid pathology. At the age of 55, approximately 50% of *APOE-ε4/ε4* individuals display abnormal levels of amyloid biomarkers, as compared to only 10% of *APOE-ε3/ε3* persons and about 20% of carriers of a single *APOE-ε4* allele.[52] Cerebral amyloid pathology has also been pinpointed as a relevant factor in the development of WMH in cognitively normal elderly individuals.[53,54] In this regard, the main limitation of our work is that we could not assess the impact in the prevalence of WMH of cerebral amyloid pathology independently of that of *APOE-ε4* status, hypertension and age.

Conclusions

Cognitively healthy, late-middle-aged *APOE-ε4* homozygotes show a higher risk of presenting pathological levels of WMH as compared to their heterozygous and non-carrier counterparts. This association was found after controlling for well-established risk factors for the development of WMH such as age and hypertension and was also independent from other CRF. Given the known association between WMH and future cognitive decline, the control of modifiable risk factors in individuals at higher risk of developing WMH appears as a useful preventive strategy to reduce or delay the onset of dementia.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The research leading to these results has received funding from "la Caixa" Foundation. Additional funding was obtained from Fondo de Investigación Sanitaria (FIS), Instituto de Salud Carlos III (ISC-III) under grant PI12/00326 and Barcelona city council under agreement #0724/13. Juan D Gispert holds a 'Ramón y Cajal' fellowship (grant no. RYC-2013-13054).

Acknowledgements

This publication is part of the ALFA study (ALzheimer and FAmilies). The authors would like to express their most sincere gratitude to the ALFA project volunteers, without whom this research would have not been possible.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors' contributions

SR and AB-R contributed equally to this work. All authors listed (SR, AB-R, NB, CM, AT, CF, AC, SM, ME, NG, KF, JC, JLM and JDG) made a substantial contribution to the concept and design, acquisition of data or analysis and interpretation of data, drafted the article or revised it critically for important intellectual content and approved the version to be published.

Supplementary material

Supplementary material for this paper can be found at the journal website: http://journals.sagepub.com/home/jcb

References

1. Sandeman EM, Hernandez Mdel C, Morris Z, et al. Incidental findings on brain MR imaging in older community-dwelling subjects are common but serious medical consequences are rare: a cohort study. *PLoS One* 2013; 8: e71467.
2. Vernooij MW, Ikram MA, Tanghe HL, et al. Incidental findings on brain MRI in the general population. *N Engl J Med* 2007; 357: 1821–1828.
3. Fazekas F, Chawluk JB, Alavi A, et al. MR signal abnormalities at 1.5 T in Alzheimer's dementia and normal aging. *AJR Am J Roentgenol* 1987; 149: 351–356.
4. Prins ND and Scheltens P. White matter hyperintensities, cognitive impairment and dementia: an update. *Nat Rev Neurol* 2015; 11: 157–165.
5. Kapeller P, Barber R, Vermeulen RJ, et al. Visual rating of age-related white matter changes on magnetic resonance imaging: scale comparison, interrater agreement, and correlations with quantitative measurements. *Stroke* 2003; 34: 441–445.
6. Bernbaum M, Menon BK, Fick G, et al. Reduced blood flow in normal white matter predicts development of leukoaraiosis. *J Cereb Blood Flow Metab* 2015; 35: 1610–1615.
7. Huisa BN, Caprihan A, Thompson J, et al. Long-term blood-brain barrier permeability changes in Binswanger disease. *Stroke* 2015; 46: 2413–2418.
8. Haller S, Kovari E, Herrmann FR, et al. Do brain T2/FLAIR white matter hyperintensities correspond to myelin loss in normal aging? A radiologic-neuropathologic correlation study. *Acta Neuropathol Commun* 2013; 1: 14.
9. Murray ME, Vemuri P, Preboske GM, et al. A quantitative postmortem MRI design sensitive to white matter hyperintensity differences and their relationship with underlying pathology. *J Neuropathol Exp Neurol* 2012; 71: 1113–1122.
10. Liao D, Cooper L, Cai J, et al. Presence and severity of cerebral white matter lesions and hypertension, its treatment, and its control. The ARIC Study. Atherosclerosis Risk in Communities Study. *Stroke* 1996; 27: 2262–2270.
11. de Leeuw FE, de Groot JC, Achten E, et al. Prevalence of cerebral white matter lesions in elderly people: a population based magnetic resonance imaging study. The Rotterdam Scan Study. *J Neurol Neurosurg Psychiatr* 2001; 70: 9–14.
12. Hopkins RO, Beck CJ, Burnett DL, et al. Prevalence of white matter hyperintensities in a young healthy population. *J Neuroimag* 2006; 16: 243–251.
13. Brickman AM, Schupf N, Manly JJ, et al. Brain morphology in older African Americans, Caribbean Hispanics, and whites from northern Manhattan. *Arch Neurol* 2008; 65: 1053–1061.
14. Gardener H, Scarmeas N, Gu Y, et al. Mediterranean diet and white matter hyperintensity volume in the Northern Manhattan Study. *Arch Neurol* 2012; 69: 251–256.
15. Tian Q, Glynn NW, Erickson KI, et al. Objective measures of physical activity, white matter integrity and cognitive status in adults over age 80. *Behav Brain Res* 2015; 284: 51–57.
16. Debette S, Beiser A, DeCarli C, et al. Association of MRI markers of vascular brain injury with incident stroke, mild cognitive impairment, dementia, and mortality: the Framingham Offspring Study. *Stroke* 2010; 41: 600–606.
17. Inzitari D, Pracucci G, Poggesi A, et al. Changes in white matter as determinant of global functional decline in older independent outpatients: three year follow-up of LADIS (leukoaraiosis and disability) study cohort. *BMJ* 2009; 339: b2477.
18. Tosto G, Zimmerman ME, Hamilton JL, et al. The effect of white matter hyperintensities on neurodegeneration in mild cognitive impairment. *Alzheimers Dement* 2015; 11: 1510–1519.
19. Brickman AM, Zahodne LB, Guzman VA, et al. Reconsidering harbingers of dementia: progression of parietal lobe white matter hyperintensities predicts Alzheimer's disease incidence. *Neurobiol Aging* 2015; 36: 27–32.

20. Kaffashian S, Dugravot A, Elbaz A, et al. Predicting cognitive decline: a dementia risk score vs. the Framingham vascular risk scores. *Neurology* 2013; 80: 1300–1306.
21. Kivipelto M, Rovio S, Ngandu T, et al. Apolipoprotein E epsilon4 magnifies lifestyle risks for dementia: a population-based study. *J Cell Mol Med* 2008; 12: 2762–2771.
22. Vuorinen M, Spulber G, Damangir S, et al. Midlife CAIDE dementia risk score and dementia-related brain changes up to 30 years later on magnetic resonance imaging. *J Alzheimers Dis* 2015; 44: 93–101.
23. Corder EH, Saunders AM, Strittmatter WJ, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* 1993; 261: 921–923.
24. Farrer LA, Cupples LA, Haines JL, et al. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. *JAMA* 1997; 278: 1349–1356.
25. Karvonen J, Kauma H, Kervinen K, et al. Apolipoprotein E polymorphism affects carotid artery atherosclerosis in smoking hypertensive men. *J Hypertens* 2002; 20: 2371–2378.
26. Schilling S, DeStefano AL, Sachdev PS, et al. APOE genotype and MRI markers of cerebrovascular disease: systematic review and meta-analysis. *Neurology* 2013; 81: 292–300.
27. Hogh P, Garde E, Mortensen EL, Jorgensen OS, et al. The apolipoprotein E epsilon4-allele and antihypertensive treatment are associated with increased risk of cerebral MRI white matter hyperintensities. *Acta Neurol Scand* 2007; 115: 248–253.
28. Godin O, Tzourio C, Maillard P, et al. Apolipoprotein E genotype is related to progression of white matter lesion load. *Stroke* 2009; 40: 3186–3190.
29. Brickman AM, Schupf N, Manly JJ, et al. APOE epsilon4 and risk for Alzheimer's disease: do regionally distributed white matter hyperintensities play a role? *Alzheimers Dement* 2014; 10: 619–629.
30. de Leeuw FE, Richard F, de Groot JC, et al. Interaction between hypertension, apoE, and cerebral white matter lesions. *Stroke* 2004; 35: 1057–1060.
31. Molinuevo JL, Gramunt N, Gispert JD, et al. The ALFA project: a research platform to identify early pathophysiological features of Alzheimer's disease. *Alzheimer's & Dementia: Translational Research & Clinical Interventions* 2016; 2: 82–92.
32. Elosua R, Garcia M, Aguilar A, et al. Validation of the Minnesota Leisure Time Physical Activity Questionnaire In Spanish Women. Investigators of the MARATDON Group. *Med Sci Sports Exerc* 2000; 32: 1431–1437.
33. Marrugat J, Vila J, Baena-Diez JM, et al. [Relative validity of the 10-year cardiovascular risk estimate in a population cohort of the REGICOR study]. *Rev Esp Cardiol* 2011; 64: 385–394.
34. Kivipelto M, Ngandu T, Laatikainen T, et al. Risk score for the prediction of dementia risk in 20 years among middle aged people: a longitudinal, population-based study. *Lancet Neurol* 2006; 5: 735–741.
35. Schmidt R, Enzinger C, Ropele S, et al. Progression of cerebral white matter lesions: 6-year results of the Austrian Stroke Prevention Study. *Lancet* 2003; 361: 2046–2048.
36. Schmidt R, Petrovic K, Ropele S, et al. Progression of leukoaraiosis and cognition. *Stroke* Sep; 38: 2619–2625.
37. Schmidt R, Schmidt H, Haybaeck J, et al. Heterogeneity in age-related white matter changes. *Acta Neuropathol* 2011; 122: 171–185.
38. Jeerakathil T, Wolf PA, Beiser A, et al. Stroke risk profile predicts white matter hyperintensity volume: the Framingham Study. *Stroke* 2004; 35: 1857–1861.
39. Homayoon N, Ropele S, Hofer E, et al. Microstructural tissue damage in normal appearing brain tissue accumulates with Framingham Stroke Risk Profile Score: magnetization transfer imaging results of the Austrian Stroke Prevention Study. *Clin Neurol Neurosurg* 2013; 115: 1317–1321.
40. Hirono N, Yasuda M, Tanimukai S, et al. Effect of the apolipoprotein E epsilon4 allele on white matter hyperintensities in dementia. *Stroke* 2000; 31: 1263–1268.
41. Habes M, Erus G, Toledo JB, et al. White matter hyperintensities and imaging patterns of brain ageing in the general population. *Brain* 2016; 139: 1164–1179.
42. Lyall DM, Munoz Maniega S, Harris SE, et al. APOE/TOMM40 genetic loci, white matter hyperintensities, and cerebral microbleeds. *Int J Stroke* 2015; 10: 1297–1300.
43. Wen HM, Baum L, Cheung WS, et al. Apolipoprotein E epsilon4 allele is associated with the volume of white matter changes in patients with lacunar infarcts. *Eur J Neurol* 2006; 13: 1216–1220.
44. Morgen K, Schneider M, Frolich L, et al. Apolipoprotein E-dependent load of white matter hyperintensities in Alzheimer's disease: a voxel-based lesion mapping study. *Alzheimers Res Ther* 2015; 7: 27.
45. Sawada H, Udaka F, Izumi Y, et al. Cerebral white matter lesions are not associated with apoE genotype but with age and female sex in Alzheimer's disease. *J Neurol Neurosurg Psychiatr* 2000; 68: 653–656.
46. Biffi A, Sonni A, Anderson CD, et al. Variants at APOE influence risk of deep and lobar intracerebral hemorrhage. *Ann Neurol* 2010; 68: 934–943.
47. Lemmens R, Görner A, Schrooten M, et al. Association of Apolipoprotein E ε2 With White Matter Disease but Not With Microbleeds. *Stroke* 2007; 38: 1185–1188.
48. DeCarli C, Reed T, Miller BL, et al. Impact of apolipoprotein E epsilon4 and vascular disease on brain morphology in men from the NHLBI twin study. *Stroke* 1999; 30: 1548–1553.
49. Dufouil C, Godin O, Chalmers J, et al. Severe cerebral white matter hyperintensities predict severe cognitive decline in patients with cerebrovascular disease history. *Stroke* 2009; 40: 2219–2221.
50. Staekenborg SS, Koedam EL, Henneman WJ, et al. Progression of mild cognitive impairment to dementia: contribution of cerebrovascular disease compared with medial temporal lobe atrophy. *Stroke* 2009; 40: 1269–1274.

51. Provenzano FA, Muraskin J, Tosto G, et al. White matter hyperintensities and cerebral amyloidosis: necessary and sufficient for clinical expression of Alzheimer disease? *JAMA Neurol* 2013; 70: 455–461.
52. Jansen WJ, Ossenkoppele R, Knol DL, et al. Prevalence of cerebral amyloid pathology in persons without dementia: a meta-analysis. *JAMA* 2015; 313: 1924–1938.
53. Marnane M, Al-Jawadi OO, Mortazavi S, et al. Periventricular hyperintensities are associated with elevated cerebral amyloid. *Neurology* 2016; 86: 535–543.
54. Scott JA, Braskie MN, Tosun D, et al. Cerebral amyloid and hypertension are independently associated with white matter lesions in elderly. *Front Aging Neurosci* 2015; 7: 221.