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Association of *APOE* ε4 with cerebral gray matter volumes in non-demented older adults: The MEMENTO cohort study

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ABSTRACT

Data on 2,045 non-demented individuals with memory complaints were drawn from the Memento cohort study to examine the association between Apolipoprotein E ε4 allele (*APOE4*) and regional brain gray matter volumes. Linear regression was used to examine the association of *APOE4* and measures of regional gray matter volumes in cross-sectional analysis and change therein using longitudinal analyses based on two brain MRI performed at baseline and at two-year follow-up. Overall, in analyses adjusted for age, sex, and intracranial volume, the presence of *APOE4* was associated with lower total gray matter volume at baseline and with a higher atrophy rate over the follow-up. The hippocampus and entorhinal cortex were the two gray matter regions most associated with *APOE4*. Further adjustment for cardiovascular risk factors had little impact on these associations. There was an interaction between age, APOE4 status and total brain volume atrophy rate, with evidence of an earlier age at onset of atrophy in hippocampal volume in *APOE4* carriers compared to non-carriers. Those results are in accordance with the role of medial temporal structures in the greater risk of dementia observed in people carrying the *APOE4* allele.

1. Introduction

Apolipoprotein E ε4 allele (*APOE4*) is known to be the strongest genetic risk factor for sporadic Alzheimer's disease (AD) dementia (Serrano-Pozo et al., 2021). *APOE* ε4 heterozygous carriers have a 3-fold higher risk of AD, ε4 homozygous carriers have up to a 15-fold higher risk, while the ε2 allele is associated with a nearly 50% lower risk of AD, compared to ε3 homozygous carriers which is the most prevalent genotype (Belloy et al., 2019). The association between *APOE4* and AD is strongest at around 65–70 years, accounting for 70% of the population attributable risk of AD in this age-group; associations are weaker both at younger and older ages (Saddiki et al., 2020). Besides AD dementia, *APOE4* carriers also have a higher risk of other dementia subtypes, including Lewy Body disease (Dickson et al., 2018), vascular dementia (Lamar et al., 2019). They also experience accelerated cognitive decline over the life course (Gharbi-Meliani et al., 2021; Hays et al., 2019; Rawle et al., 2018).

The mechanisms underlying the association between *APOE4* and AD are complex and remain poorly understood (Mahoney-Sanchez et al., 2016; Serrano-Pozo et al., 2021). *APOE4* is thought to play a role in de-

Abbreviations: Aβ, beta-amyloid peptide; AD, Alzheimer's disease; APOE4, apolipoprotein E ε4 allele; IPW, inverse probability weighting; MMSE, mini-mental state examination; SE, standard error; WMH, white matter hyperintensities.

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creased clearance of beta-amyloid peptide (Aβ) (Kanekiyo et al., 2014), hyperphosphorylation of tau protein (Wadhwani et al., 2019), and may also have direct toxic effect on neurons (Simonovitch et al., 2016). Further, *APOE4* also affects atherosclerosis susceptibility (Zhu et al., 2016) and increases risk of cardiovascular events (Xu et al., 2016), resulting in its association with AD dementia being partly mediated by cerebrovascular mechanisms (DeCarli et al., 1999).

Quantitative brain MRI volumetry data can provide important insights into understanding the association between *APOE4* and cognitive outcomes by identifying areas of gray matter most susceptible to the adverse effects of the ε4 allele. Most of the research in this domain is either cross-sectional (Burggren et al., 2008), based on small studies (Lu et al., 2011), or only includes individuals at a late stage of disease (Drzezga et al., 2009). The association between *APOE4* and gray matter volume in non-demented persons has been examined in few studies and the results are inconsistent (Cherbuin et al., 2008; Squarzoni et al., 2018). To address some of these limitations, we examined the association between APOE4 status and regional volumes of gray matter in cross-sectional and longitudinal analyses using data from a large, multicenter cohort of non-demented individuals with memory complaints (Dauphinot et al., 2020). We hypothesized that *APOE4* carriers would have smaller gray matter volumes and higher atrophy rates in the medial temporal lobe of the brain, known to be involved early in the neuropathological process of AD. We examined whether these associations were independent of age and cardiovascular risk factors.

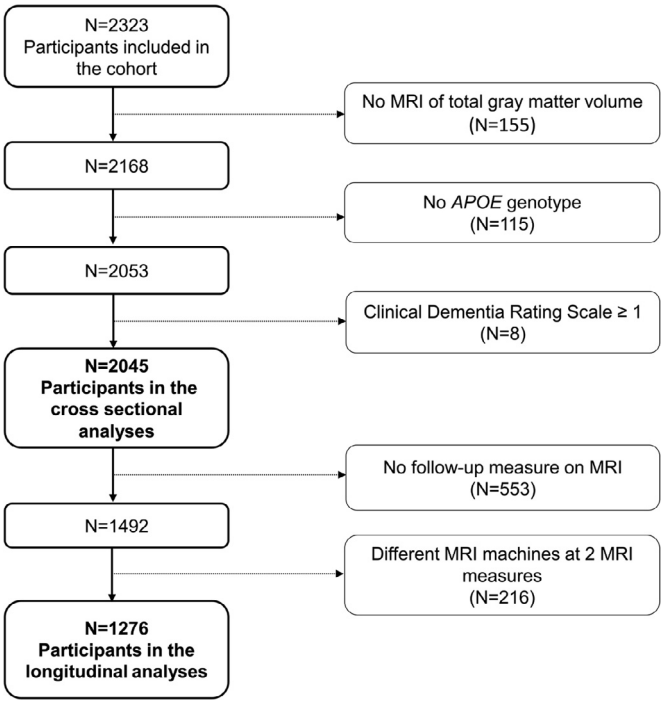

Fig. 1. Flow-chart of the study.

2. Methods

2.1. Study population

Data are drawn from the MEMENTO cohort study, a prospective cohort of 2323 non-demented persons recruited from April 2011 to June 2014 by a French national network of 26 university-based memory clinics (Dufouil et al., 2017). The inclusion criteria were as follows: age ≥ 18 years, and mild cognitive impairment or cognitive complaints. Mild cognitive impairment was defined as (1) performance on cognitive tests in the 6 months preceding recruitment to the study that were 1 standard deviation below those compared to persons matched for age, sex, and education; (2) a Clinical Dementia Rating (CDR) ≤0.5 and not having a dementia diagnosis (Albert et al., 2011). Details of the neuropsychological test battery and the age, sex and education norms for each test are available in a previous publication (Dufouil et al., 2017). Exclusion criteria were as follows: vulnerable adults under "guardianship"; residents of nursing facilities; pregnant or breastfeeding women; AD known to be caused by gene mutations; history of intracranial surgery; neurological disease such as treated epilepsy, Parkinson's disease, Huntington's disease, brain tumor, subdural haematoma, progressive supranuclear palsy, or history of head trauma or stroke followed by persistent neurological deficits; stroke diagnosed in the last 3 months preceding recruitment; schizophrenia diagnosis; and illiteracy (not able to count or read).

In the present study, we restricted the analyses to participants with data on brain MRI data and *APOE* genotype at baseline (n = 2045). Of these participants, 1276 had a second brain MRI approximately 2 years later; a flow-chart of the study is presented in Fig. 1.

2.2. Standard protocol approvals, registrations, and patient consents

This study was performed in accordance with the Declaration of Helsinki. All participants provided written informed consent. The MEMENTO cohort protocol was approved by the local ethics committee ("Comité de Protection des Personnes Sud-Ouest et Outre Mer III"; approval number 2010-A01394-35) and registered in ClinicalTrials.gov (Identifier: NCT01926249).

2.3. Brain morphometry

Brain MRI acquisition from all centres were curated by the "Center d'Acquisition et de Traitement des Images" (CATI), a national platform dedicated to neuroimaging (http://cati-neuroimaging.com) using a systematic qualification procedure to ensure parameter uniformity and image quality (Operto et al., 2016). The 3D T1 weighted images were acquired in the sagittal plane, with 1 mm slices for 3T systems and 1.3 mm slices for 1.5T systems, lasting nine to ten minutes. The resolution was isotropic in the acquisition plane (here sagittal), so the voxels were 1mmx1mmx1mm in the sagittal plane. Acceleration or averaging was not used. Software versions were from VB15 to VE11 for Siemens systems, from v2.6 to v5.1 for Philips systems and from HD15 to HD 25 for GE systems. Further details are provided in Supplementary Table 1.

2.4. Gray matter volumetry

Whole-brain and regional gray matter volumes were calculated using three dimensional (3D) T1-weighted acquisition with 1 mm isotropic resolution using the ADNI study protocol, with the FreeSurfer software and ROIs of the Desikan-Killiany atlas (Desikan et al., 2006). All the 3D T1 weighted images were processed independently with the same default settings using the "reconall" pipeline of FreeSurfer software (Fischl et al., 2002) to obtain regions of interest used in volume-based analyses. Since a follow-up image was available for most subjects, the longitudinal processing pipeline was used (Reuter et al., 2012). This pipeline creates an unbiased within-subject template space together with a template 3D dataset; several processing steps are then initialized with common information from this within-subject template. It allows extracting, among other things, total gray matter volume and whole or regional cortical volumes as well as hippocampal volumes. The cortical ROIs were extracted using the Desikan-Kiliany atlas (Desikan et al., 2006); the list of gray matter regions available in the study is presented in Supplementary Table 2. Total intra cranial volume (TIV) was obtained with SPM processes: all the 3D T1 were thus also processed by the "Segment" module of SPM12 software (Ashburner and Friston, 2005) with the default tissue probability maps (TPM) template. This module clas-

sifies the brain into gray matter, white matter and cerebral-spinal fluid probability maps and allows us to estimate a robust TIV for each dataset.

The volume of white matter hyperintensities (WMH) was measured by the WHASA software (White matter Hyperintensities Automated Segmentation Algorithm), a method designed for automatically segmenting white matter hyperintensities from FLAIR and 3D T1-weighted images (Samaille et al., 2012). All the segmentation results were monitored by visual inspection using specific snapshots according to a predefined procedure and scored on a scale from 0 (failure) to 4 (perfect segmentation): the data from 32 participants could not be analyzed due to complete failure (grade 0) and 32 participants were excluded due to poor results (grade 1). For the follow-up data, the data from 26 participants could not be analyzed due to complete failure and 17 participants were excluded because of poor results.

2.5. APOE genotyping

DNA was extracted from peripheral blood samples using Gentra Puregene blood kits (QIAGEN, Hilden, Germany). Apolipoprotein E genotypes were determined by KBiosciences (Hoddesdon, UK; www.kbioscience.co.uk), using fluorescence-based competitive allele-specific polymerase chain reaction. Two *APOE* single-nucleotide polymorphisms, rs429358 and rs7412, allowed identification of the three major APOE alleles ($\varepsilon 2$, $\varepsilon 3$ and $\varepsilon 4$) (Dufouil et al., 2017).

2.6. Covariates

Sociodemographic variables included age, sex, and education (3 categories: no education to primary school, secondary school to high school, baccalaureate or university degree).

The Mini-Mental State Examination (MMSE), a 30-item brief cognitive test.

Depression was assessed using the depression/dysphoria subscale score of the Neuropsychiatric Inventory-Clinician rating scale (NPI-C), with depression denoted by a score greater or equal to 2.

Cardiovascular risk factors included smoking habits, hypertension (systolic/diastolic blood pressure ≥140(90) mmHg or use of antihypertensive drugs), LDL cholesterol level, diabetes mellitus (use of medication for diabetes or fasting glucose>7.0mmol/l), and hypercholesterolemia (total cholesterol ≥6.2mmol/l or use of lipid lowering drugs).

2.7. Statistical analysis

Baseline characteristics were examined in the total cohort and by *APOE4* status ($\varepsilon 4$ carriers vs non-carriers). Comparisons between groups were examined using a χ^2 test for categorical variables and analysis of variance for continuous variables. A $\chi 2$ test was used to examine the Hardy-Weinberg equilibrium in the distribution of *APOE* genotype in the study population.

Cross-sectional analyses

Linear regression was used to examine the cross-sectional association between *APOE4* status and regional gray matter volumes. To compare effect size, we used relative difference (percentage), defined as the beta coefficient associated with *APOE4* in the linear regression divided by the mean value of the corresponding volume and multiplied by 100.

The analyses were first adjusted for total intracranial volume, age, age^2, sex, depression, volume of WMH, MRI machine and interaction term between age and *APOE* (model 1). Then cardiovascular risk factors (hypercholesterolemia, LDL cholesterol, smoking, diabetes mellitus, hypertension, BMI) were added to the analyses (model 2). To quantify the impact of adjustment for cardiovascular risk factors the percentage change between models 1 and 2 was defined as: $[(\beta_2\text{-}\beta_1)/\ \beta_1] * 100$.

Longitudinal analyses

To examine the association between *APOE4* status and change in gray matter volumes, linear regression was used with gray matter volumes at MRI follow-up as dependent variable and *APOE4* status, MRI volumes at baseline, time (in years) between the 2 MRI scans, and covariates as independent variables. The analyses were first adjusted for total intracranial volume (TIV), age, age^2, sex, depression, WMH volume, and MRI machine. The estimates were ranked in importance using percentage of change between the two MRI measures, defined as 100 x *APOE4* beta coefficient in the linear regression described above divided by the mean value of the corresponding volume at baseline. As there was a significant interaction of *APOE4* with age and age^2, both these interaction terms were included in the analyses and results were reported for mean age (model 1). As with the cross-sectional analysis, cardiovascular risk factors were added in the second step in the analyses.

The second MRI measure was available on 1276 of the 2045 participants included at baseline leading us to use inverse probability weighting (IPW) to take loss to follow-up into account. This involved first calculating the probability of being present at the second MRI measure using baseline data on regional gray matter volume, *APOE*, age, sex, TIV, depression, WMH volume, education, and the MMSE score. Then the inverse of these probabilities was used to weight the analyses of change in gray matter volumes.

Sensitivity analysis

Due to the small number of *APOE4* homozygotes in the study population, the main analyses were based on a dichotomous *APOE4* measure ($\varepsilon 4$ carriers vs non-carriers). In sensitivity analysis, we repeated the cross-sectional and longitudinal analyses using more detailed categorization of *APOE* genotypes (5 categories: $\varepsilon 2\varepsilon 2$/ $\varepsilon 2\varepsilon 3$; $\varepsilon 3\varepsilon 3$; $\varepsilon 2\varepsilon 4$; $\varepsilon 3\varepsilon 4$; $\varepsilon 4\varepsilon 4$). We also reran the analyses after excluding *APOE4* homozygous individuals to examine the impact on results.

There was no evidence of an effect of laterality for the association between *APOE4* and regional gray matter volumes (all p values > 0.30) leading us to use mean value of left/right hemisphere volumes in the main analyses but separate analyses in the right and left hemisphere were also undertaken.

Bonferroni correction was applied to correct for multiple comparisons and we retained two-tailed values of $p < 0.0025$ to be statistically significant. Analyses were performed using Stata 15 (StataCorp LP, College Station, TX).

3. Results

3.1. Demographic characteristics

A total of 2323 participants were included in the Memento cohort study. Among them, 278 were excluded from the present analysis due to missing or insufficient quality data on brain MRI (n = 123), missing *APOE* genotype (n = 115)) and prevalent dementia (Clinical Dementia Rating Scale=1 (n = 8)). A total of 2045 participants were included in the cross-sectional analyses (flow-chart, Fig. 1).

Table 1 summarizes participants' baseline characteristics, overall and by *APOE4* status. The mean (SD) age of participants was 70.9 (8.6) years (range: 34.7 to 93.1), the median (interquartile) age was 71.7 (65.6;77.2) years. Women represented 62% of participants included in the analysis. The frequency of the $\varepsilon 3$, $\varepsilon 4$ and $\varepsilon 2$ alleles was 77%, 16.5%, and 6.5% respectively. The distribution of *APOE* genotype was consistent with Hardy-Weinberg equilibrium (P = 0.50). Sixty percent of the study population were *APOE* $\varepsilon 3/\varepsilon 3$ carriers, and approximately 30% carried at least one $\varepsilon 4$ allele (heterozygotes: 26%, homozygotes: 3%), and 10% were either $\varepsilon 2/\varepsilon 2$ (0.4%) or $\varepsilon 2/\varepsilon 3$ (10.2%). Compared to non-$\varepsilon 4$ carriers, participants with at least one $\varepsilon 4$ allele were younger, more likely to be men, had a lower MMSE score, and were more likely to have hypercholesterolemia.

3.2. Cross sectional analysis: association between APOE4 and baseline gray matter volumes

Table 2 shows the cross-sectional association between *APOE4* status and regional gray matter volumes, organized according to the effect size

Table 1
Baseline characteristics of the study population.

Characteristics	Overall (N = 2045)	No *APOE4* ≥ 1 *APOE4* (N=1437)	(N=608)	P value
Age, year, mean (SD)	70.9 (8.6)	71.2 (8.6)	70.3 (8.5)	0.042
Women, n (%)	1266 (61.9)	910 (63.3)	356 (58.5)	0.042
Education, n (%)				
Low	249 (12.2)	180 (12.5)	69 (11.4)	
Medium	955 (46.8)	662 (46.1)	293 (48.3)	
High	837 (41.0)	593 (41.3)	244 (40.3)	0.60
MMSE score, mean (SD)	27.9 (1.9)	28.1 (1.8)	27.6 (2.2)	<0.001
Depression, n (%)	710 (34.7)	487 (33.9)	223 (36.7)	0.23
BMI, kg/m^2, mean (SD)	25.6 (4.3)	25.6 (4.2)	25.6 (4.4)	0.99
Hypertension, n (%)	1232 (61.42)	877 (62.11)	355 (59.76)	0.32
Hypercholesterolemia, n (%)	925 (45.23)	598 (41.61)	327 (53.78)	<0.001
Total cholesterol, mmol/L, mean (SD)	5.65 (1,11)	5.64 (1.11)	5.66 (1.12)	0.61
LDL cholesterol, mmol/L, mean (SD)	3.38 (0.95)	3.34 (0.95)	3.47 (0.97)	0.006
HDL cholesterol, mmol/L, mean (SD)	1.69 (0.47)	1.72 (0.49)	1.63 (0.42)	<0.001
Diabetes mellitus, n (%)	185 (9.10)	144 (10.08)	41 (6.79)	0.018
Blood glucose level, mmol/L, mean (SD)	5.44 (1.77)	5.47 (1.84)	5.39 (1.60)	0.35
Former or current smokers, n (%)	851 (41.74)	588 (41.03)	263 (43.40)	0.32
White matter hyperintensities, cm^3, mean (SD)	9.45 (12.1)	9.25 (11.9)	9.90 (12.5)	0.28
APOE genotype, n (%)				
$\varepsilon2/\varepsilon2$	8 (0.4)			
$\varepsilon2/\varepsilon3$	209 (10.2)			
$\varepsilon3/\varepsilon3$	1220 (59.7)			
$\varepsilon2/\varepsilon4$	40 (2.0)			
$\varepsilon3/\varepsilon4$	500 (24.4)			
$\varepsilon4/\varepsilon4$	68 (3.3)			

Table 2
Cross-sectional association between *APOE4* status and regional gray matter volumes, results show top 20 volumes sorted by relative difference. P-values highlighted in bold are significant below the Bonferroni corrected threshold 0.0025.

			Difference in volumes (≥ 1 *APOE4* vs No *APOE4*)					
			Model 1*			Model 2†		
Brain volumes, cm^3, mean (SD)	No *APOE4* (*N* = 1437)	≥ 1 *APOE4* (*N* = 608)	β_1 (SE)	Relative difference (%)‡	P value	β_2 (SE)	P value	$\Delta\beta$ (%)§
Total gray matter volume	576.3 (74.6)	575.2 (75.3)	−8.029 (2.492)	−1.4	0.001	−7.482 (2.416)	0.002	6.8
Entorhinal cortex	1.63 (0.33)	1.61 (0.32)	−0.046 (0.014)	−2.8	0.001	−0.046 (0.015)	0.002	−0.43
Hippocampus	2.72 (0.38)	2.70 (0.40)	−0.056 (0.016)	−2.1	0.001	−0.059 (0.016)	0.001	−4.8
Inferior temporal gyrus	9.29 (1.5)	9.25 (1.5)	−0.181 (0.059)	−2.0	0.002	−0.187 (0.060)	0.002	−3.7
Parahippocampal gyrus	1.96 (0.31)	1.95 (0.32)	−0.032 (0.013)	−1.7	0.01	−0.034 (0.013)	0.01	−4.0
Inferior parietal lobule	11.9 (1.7)	11.8 (1.6)	−0.153 (0.064)	−1.3	0.02	−0.193 (0.065)	0.003	–
Superior temporal sulcus	2.19 (0.34)	2.18 (0.35)	−0.028 (0.014)	−1.3	0.048	−0.037 (0.014)	0.009	–
Isthmus of the cingulate gyrus	2.27 (0.37)	2.26 (0.35)	−0.027 (0.014)	−1.2	0.05	−0.030 (0.014)	0.03	–
Putamen	4.76 (0.72)	4.76 (0.72)	−0.054 (0.031)	−1.1	0.08	−0.064 (0.032)	0.04	–
Superior temporal gyrus	10.4 (1.5)	10.4 (1.5)	−0.095 (0.050)	−0.91	0.06	−0.102 (0.051)	0.046	–
Supramarginal gyrus	9.32 (1.3)	9.33 (1.2)	−0.081 (0.048)	−0.87	0.09	−0.106 (0.049)	0.03	–
Cerebellar	45.4 (5.5)	45.6 (5.4)	−0.377 (0.204)	−0.83	0.06	−0.523 (0.208)	0.01	–
Frontal pole	0.78 (0.14)	0.77 (0.13)	−0.006 (0.006)	−0.77	0.35	−0.008 (0.007)	0.22	–
Thalamus	6.49 (0.96)	6.49 (0.91)	−0.048 (0.032)	−0.75	0.13	−0.057 (0.033)	0.09	–
Precuneus	8.55 (1.1)	8.55 (1.1)	−0.060 (0.042)	−0.7	0.16	−0.074 (0.043)	0.09	–
Middle temporal gyrus	9.76 (1.4)	9.80 (1.4)	−0.064 (0.053)	−0.65	0.22	−0.080 (0.053)	0.13	–
Inferior frontal gyrus	3.28 (0.51)	3.29 (0.51)	−0.021 (0.023)	−0.65	0.34	−0.032 (0.023)	0.18	–
Lateral orbitofrontal cortex	6.62 (0.77)	6.65 (0.85)	−0.041 (0.030)	−0.62	0.17	−0.043 (0.031)	0.17	–
Lingual gyrus	5.93 (0.92)	5.94 (0.90)	−0.029 (0.039)	−0.49	0.45	−0.029 (0.040)	0.46	–
Posterior cingulate cortex	2.84 (0.41)	2.85 (0.37)	−0.013 (0.016)	−0.47	0.39	−0.016 (0.016)	0.31	–
Insula	6.34 (0.77)	6.37 (0.81)	−0.025 (0.030)	−0.39	0.41	−0.029 (0.031)	0.34	–

* Adjusted for total intracranial volume, age, age^2, sex, MRI machine, depression, volume of WMH and interaction between *APOE* and age.
† Model 1 + dyslipidemia, LDL cholesterol, diabetes, hypertension, BMI and smoking status.
‡ Relative difference (%) = (β_1/mean volume)x100.
§ $\Delta\beta$ (%) = ((β_1-β_2)/β_1) x 100.

of the associations. In analyses adjusted for total intracranial volume, age, age^2, sex, depression, WMH volume, interaction between age and *APOE* (model 1), *APOE4* carriers had a lower total gray matter volume compared to non-carriers. Hippocampus and entorhinal cortex volumes had the strongest associations with *APOE4*. Adjustment for cardiovascular risk factors (Table 2, model 2) had little impact on the association between *APOE* $\varepsilon4$ and hippocampus ($\Delta\beta$= −4.8%) and entorhinal cortex ($\Delta\beta$= −0.43%) volumes. There were no sex differences in the association between *APOE4* and brain volumes, for total gray matter volume (p for interaction = 0.52, model 2) or for any of the regional gray matter volumes (all p values > 0.30). Cross-sectional associations for the left (supplementary Table 3) and right (supplementary Table 4) hemispheres were similar.

Analyses based on 5 categories of *APOE* genotypes are shown in Supplementary Table 5. Compared to $\varepsilon3/\ \varepsilon3$ participants, $\varepsilon2\varepsilon2/\ \varepsilon2\varepsilon3$ carriers had similar total gray matter volume and other regional gray mat-

Table 3
Longitudinal association between APOE4 status and annual change in regional gray matter volumes; results show top 20 volumes sorted by percentage of change. P-values highlighted in bold are significant below the Bonferroni corrected threshold 0.0025.

	Difference in volume at follow-up (≥1 *APOE4* vs No *APOE4*)					
	Model 1*			Model 2†		
Brain volumes, cm^3	β_1 (SE)	% change‡	P value	β_2 (SE)	P value	$\Delta\beta$ (%)§
Total gray matter volume	−10.32 (3.58)	−1.8	0.004	−9.87 (3.91)	0.01	4.4
Hippocampus	−0.044 (0.011)	−1.6	<0.001	−0.040 (0.011)	<0.001	9.6
Temporal pole	−0.034 (0.010)	−1.5	0.001	−0.037 (0.011)	0.001	−10.4
Entorhinal cortex	−0.023 (0.009)	−1.4	<0.001	−0.019 (0.010)	<0.001	15.3
Inferior temporal gyrus	−0.132 (0.029)	−1.4	0.01	−0.128 (0.030)	0.04	3.2
Middle temporal gyrus	−0.137 (0.029)	−1.4	<0.001	−0.144 (0.031)	<0.001	−4.8
Inferior parietal lobule	−0.164 (0.038)	−1.4	<0.001	−0.174 (0.040)	<0.001	−5.8
Fusiform gyrus	−0.116 (0.025)	−1.3	<0.001	−0.115 (0.027)	<0.001	0.8
Precuneus	−0.106 (0.026)	−1.2	<0.001	−0.119 (0.027)	<0.001	−12.3
Parahippocampal gyrus	−0.023 (0.006)	−1.2	<0.001	−0.023 (0.006)	<0.001	3.1
Superior temporal sulcus	−0.026 (0.007)	−1.2	<0.001	−0.027 (0.007)	<0.001	−3.6
Supramarginal gyrus	−0.104 (0.026)	−1.1	<0.001	−0.109 (0.027)	<0.001	−5.4
Isthmus of the cingulate gyrus	−0.025 (0.006)	−1.1	<0.001	−0.023 (0.006)	<0.001	10.1
Transverse occipital sulcus	−0.105 (0.031)	−1.0	<0.001	−0.107 (0.033)	0.001	−2.2
Posterior cingulate cortex	−0.029 (0.007)	−1.0	<0.001	−0.031 (0.007)	<0.001	0.4
Putamen	−0.049 (0.017)	−1.0	0.004	−0.049 (0.016	0.002	−6.3
Superior parietal lobule	−0.117 (0.040)	−0.99	0.003	−0.138 (0.041)	0.001	−17.9
Caudal middle frontal gyrus	−0.052 (0.017)	−0.95	0.002	−0.059 (0.018)	0.001	−13.8
Insula	−0.058 (0.013)	−0.91	<0.001	−0.059 (0.014)	<0.001	−2
Inferior frontal gyrus	−0.018 (0.006)	−0.87	0.005	−0.018 (0.007)	0.01	0.7
Rostral middle frontal gyrus	−0.117 (0.044)	−0.86	0.007	−0.131 (0.046)	0.004	−12

* Adjusted for baseline volume and total intracranial volume, age, age^2, sex, MRI machine, depression, WMH, time between the 2 MRI, and interactions between *APOE*xagexage^2.
† Model 1 + dyslipidemia, LDL cholesterol, diabetes, hypertension, BMI and smoking status.
‡ % change = β_1/mean volume at baseline.
§ $\Delta\beta$ (%) = $((\beta_1-\beta_2)/\beta_1)$ x 100.

ter volumes. Participants with $\varepsilon3/\varepsilon4$ and $\varepsilon4/\varepsilon4$ genotypes had lower gray matter volumes, with the effect estimate being largest in the $\varepsilon4/\varepsilon4$ group.

3.3. *Longitudinal analysis: association between* APOE4 *and change in gray matter*

A total of 1276 participants who had a second MRI measure after a 2.1-year median follow-up were included in the longitudinal analyses (Fig. 1). Compared to those with a second brain MRI assessment, participants without such data were older, had lower education and lower mean MMSE scores but did not differ on prevalence of *APOE* $\varepsilon4$ allele (Supplementary Table 6).

Table 3 presents the longitudinal associations between *APOE4* status and the annual change in gray matter volumes over the follow-up, in models using IPW to account for missing MRI data. After adjustment for intracranial volume, age, age^2, sex, depression, WMH volume, and interactions of *APOE* with age and age^2 (Model 1), *APOE4* carriers had greater atrophy in total gray matter volume. *APOE4* carriers also had greater atrophy in hippocampus, temporal pole, and entorhinal cortex volumes. Further adjustment for cardiovascular risk factors (Table 3, model 2) had little impact on these associations. Our analyses did not show sex differences in the longitudinal association between *APOE4* and brain volumes, either for total gray matter volume (p for interaction = 0.53, model 2) or the regional gray matter volumes (all p values >0.30).

The longitudinal associations for volumes in the left (supplementary Table 7) and right (supplementary Table 8) hemispheres were similar to results in the main analysis. Analyses based on 5 categories of *APOE* genotypes are shown in Supplementary Table 9. Compared to $\varepsilon3/\varepsilon3$ participants, $\varepsilon2\varepsilon2$/ $\varepsilon2\varepsilon3$ carriers had a similar decline in total gray matter volume and regional gray matter volume. Participants with $\varepsilon3/\varepsilon4$ and $\varepsilon4/\varepsilon4$ genotypes had higher decline in gray matter volumes over the follow-up.

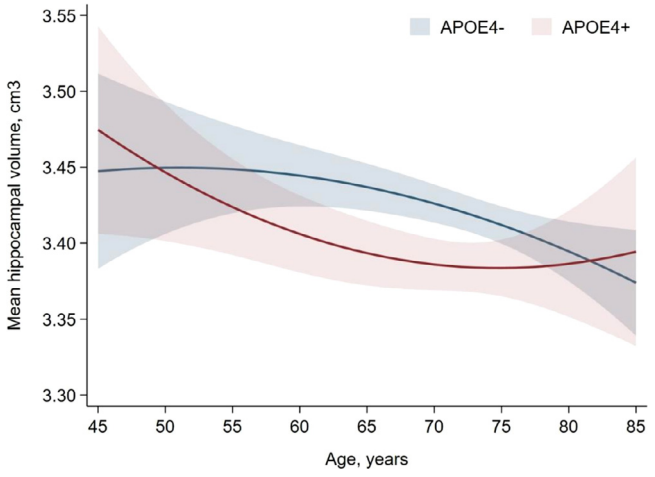

Fig. 2. Change in hippocampal volume at 2-year follow-up as a function of age in *APOE4* carriers and non-carriers. Linear regression models are adjusted for baseline volume and total intracranial volume, age, age^2, sex, MRI machine, depression, white matter hyperintensities, time between the 2 MRI, interactions between *APOE*, age, and age^2, dyslipidemia, LDL cholesterol, diabetes, hypertension, BMI, and smoking status. 95% confidence intervals around the estimate are shown by the shaded area in the figure. Predictions were made for a fixed baseline volume of 3.5 cm^3.

3.4. *Interaction between age and* APOE4

There was a significant interaction between age and *APOE4* for associations with change in gray matter volume (p for interaction = 0.036). Fig. 2 shows this interaction for hippocampal volume, with larger difference in hippocampal volume between *APOE4* status observed between age 60 and 75 years.

3.5. Sensitivity analysis: exclusion of APOE4 homozygous

Repeating the analyses after excluding 68 *APOE4* homozygous participants attenuated the associations but results were broadly similar, both in cross-sectional (Supplementary Table 10) and longitudinal (Supplementary Table 11) analyses.

4. Discussion

In this large, multi-center study of over 2000 older adults free of dementia, we found *APOE* ε4 allele to be associated with lower gray matter volume and higher gray matter atrophy over the two-year follow-up. In analyses of regional gray matter volumes, we found hippocampus and the entorhinal cortex volumes to be the most susceptible areas, both in cross-sectional and longitudinal analyses. As *APOE4* has been shown to be associated with risk of cardiovascular disease and atherosclerosis, we examined the role of cardiovascular risk factors in the association between *APOE4* and gray matter volumes and found little impact of these factors, suggesting that the association between *APOE* and gray matter volume is likely to be driven by neurodegenerative rather than vascular processes.

Although *APOE4* allele is a major genetic risk factor for AD dementia and accelerated cognitive decline in older adults, few studies have examined associations with gray matter volume in individuals without a dementia diagnosis. The results of such studies are not consistent, some studies reported an association (Cacciaglia et al., 2018; Moffat et al., 2000; Reiter et al., 2017) while others did not find an association (Burggren et al., 2008; Mondadori et al., 2007; Protas et al., 2013). Compared to previous studies, the present study is based on a larger study population with automated determination of regional gray matter volumes, allowing us to show that *APOE4* heterozygotes and not only homozygotes had lower gray matter volumes compared to non-carriers. A further feature of our study is the examination of both cross-sectional and longitudinal associations. We also used a statistical approach that allowed us to compare the strength of the association between *APOE4* and the regional gray matter volumes irrespective of the heterogeneity in volume size. These results from cross-sectional and longitudinal analyses support our hypothesis that the medial temporal structures (hippocampus and entorhinal cortex) are most susceptible to the presence of the *APOE4* allele.

Evidence of an association between *APOE4* and gray matter volumes in a population of persons without dementia diagnosis after referral to memory clinics on the basis of subjective memory complaints supports the hypothesis of wider effects of *APOE* on brain structures. A part of this effect may be explained by some individuals being at a prodromal stage of AD who might develop dementia in the future. However this might not totally explain the association as exclusion of long term incident cases of dementia in previous longitudinal studies did not fully attenuate the association between *APOE4* and faster cognitive decline in studies based on the general population (Gharbi-Meliani et al., 2021). In addition, *APOE4* has been shown to be associated not only with AD, but also others causes of dementia, including Lewy Body disease (Dickson et al., 2018) and vascular dementia (Lamar et al., 2019). Some recent findings suggest that *APOE4* carriers may have a lower age at onset of genetic forms of frontotemporal lobar dementia, independently of Aβ pathology (Koriath et al., 2019).

The mechanisms underlying the association between *APOE* and AD remain unclear, although the beta-amyloid peptide pathway is thought to be important. Neuropathological studies consistently find an association between *APOE* ε4 and higher Aβ plaque burden and more severe cerebral amyloid angiopathy (Serrano-Pozo et al., 2015), is also observed in PET amyloid studies (Fleisher et al., 2013; Jansen et al., 2015). Previous studies show that *APOE4* promotes the seeding of Aβ peptide into Aβ oligomers and fibrils (Hori et al., 2015), inhibit Aβ clearance from the brain (Castellano et al., 2011) and decrease its enzymatic degradation (Deane et al., 2008). Recently, PET tau studies have also reported an association between *APOE4* and the presence of tau pathology, particularly in the entorhinal cortex and hippocampus, independently of Aβ deposits (Therriault et al., 2020). Beyond its association with AD neuropathological hallmarks, some recent studies have also found an association of *APOE4* with the presence and severity of other complex proteinopathies accumulation including TDP-43 pathology (Wennberg et al., 2018), alpha-synuclein deposits (Robinson et al., 2018) or cerebrovascular lesions (Montagne et al., 2020; Williams et al., 2019).

Several previous studies suggest that women are more vulnerable to the deleterious effect of *APOE4* (Neu et al., 2017; Ungar et al., 2014), but this associations remains the subject of debate (Yamazaki et al., 2019). Our previous research in a large multi-center case controls study showed no sex differences in the association of *APOE* and AD, defined using with cerebrospinal fluid biomarkers, once age was carefully taken into consideration (Saddiki et al., 2020). In the present study, we did not find sex differences in the association between *APOE4* and gray matter volumes, in cross-sectional or longitudinal analyses. Future studies, incorporating both biological and radiological biomarkers are needed to better understand the complex relationship between AD risk, sex, and *APOE4* in the population.

4.1. Strengths and limitations

This study has several strengths, including the centralized harmonization of brain MRI data and the large sample size, particularly in the longitudinal analyses. The use of IPW to take attrition into account in these analyses is likely to reduce bias in the estimates (Weuve et al., 2015). Use of Bonferonni correction allowed us to take multiple testing into account. These findings need to be considered in light of some limitations. As there was only 2 MRI scans available per participant, we were not able to use more sophisticated statistical analyses (Sørensen et al., 2021). Participants included in this study were drawn from referrals to memory centers, and although they did not have clinical dementia they were referred on the basis of subjective memory complaints and may therefore not be representative of the general population of older adults.

4.2. Conclusion

In conclusion, we found that gray matter volumes localized in the medial temporal area were more strongly associated with *APOE4* in non-demented older adults, independently of cardiovascular risk factors. Differences in the rate of gray matter atrophy were observed compared to non-carriers. These findings highlight the importance of early interventions, if and when available, to mitigate the effect of *APOE4*.

Data and code availability

Anonymized data can be shared on request with any qualified investigator for the sole purpose of replicating procedures and results presented in the article, provided data transfer is consistent with EU legislation on the general data protection regulation.

Credit authorship contribution statement

Mélina Régy: Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing. **Aline Dugravot:** Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing. **Séverine Sabia:** Conceptualization, Formal analysis, Writing – review & editing. **Aurore Fayosse:** Data curation, Writing – review & editing. **Jean-Francois Mangin:** Data curation, Writing – review & editing. **Marie Chupin:** Data curation, Writing – review & editing. **Clara Fischer:** Data curation, Writing – review & editing. **Vincent Bouteloup:** Data curation, Writing – review & editing. **Carole Dufouil:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition. **Geneviève Chêne:** Conceptualization, Writing – review & editing,

Funding acquisition. **Claire Paquet:** Conceptualization, Writing – review & editing. **Bernard Hanseeuw:** Conceptualization, Writing – review & editing. **Archana Singh-Manoux:** Conceptualization, Writing – original draft, Writing – review & editing. **Julien Dumurgier:** Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing, Supervision.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.neuroimage.2022.118966.

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