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FEATURED ARTICLE

# The protective gene dose effect of the *APOE ε2* allele on gray matter volume in cognitively unimpaired individuals

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## Abstract

**Introduction:** Harboring two copies of the apolipoprotein E (*APOE*) *ε2* allele strongly protects against Alzheimer's disease (AD). However, the effect of this genotype on gray matter (GM) volume in cognitively unimpaired individuals has not yet been described.

**Methods:** Multicenter brain magnetic resonance images (MRIs) from cognitively unimpaired *ε2* homozygotes were matched (1:1) against all other *APOE* genotypes for relevant confounders (n = 223). GM volumes of *ε2* genotypic groups were compared to each other and to the reference group (*APOE ε3/ε3*).

**Results:** Carrying at least one *ε2* allele was associated with larger GM volumes in brain areas typically affected by AD and also in areas associated with cognitive resilience. *APOE ε2* homozygotes, but not *APOE ε2* heterozygotes, showed larger GM volumes in areas related to successful aging.

**Discussion:** In addition to the known resistance against amyloid-*β* deposition, the larger GM volumes in key brain regions may confer *APOE ε2* homozygotes additional protection against AD-related cognitive decline.

**KEYWORDS**
Alzheimer's disease, Alzheimer's disease signature, apolipoprotein E *ε2* carrier, brain maintenance, brain morphology, brain reserve, cognitive reserve, magnetic resonance, multi-site, resilience signature

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## 1 | BACKGROUND

The apolipoprotein E (*APOE*) gene is the major genetic risk-modifying factor for sporadic Alzheimer's disease (AD). Carrying one or two copies of the ε4 allele confers higher risk for AD (allelic dose odds ratio [OR]: 6), whereas carrying the ε2 allele confers lower risk for AD (allelic dose OR: 0.38).[1–3] The increased AD risk as a consequence of carrying at least one ε4 allele has been primarily related to a higher amyloid-β (Aβ) burden in the brain, in a dose dependent manner (*i.e.*, number of ε4 alleles).[4] However, neuroimaging studies have shown a relationship between ε4 gene dose and lower brain glucose hypometabolism and smaller gray matter (GM) volumes in AD-related brain areas,[5,6] even in cognitively unimpaired individuals. These findings suggest an ε4 gene dose reduced capacity to maintain brain health.[7]

On the other hand, the *ε2* allele has received much less attention, presumably due to the low frequency of this polymorphism in the general population (8.4%).[8] *APOE* ε2 carriers have lower Aβ burden among non-demented participants.[9,10] However, multiple studies suggest that the ε2 allele may reduce the risk of AD through Aβ-independent pathways.[11] One of these pathways may be through maintained GM volumes across the lifespan. In healthy adolescents no differences between ε2 and ε4 carriers or dose dependent effects of these alleles were found in hippocampal volumes.[12] However, potential gene dose effects of the ε2 allele in adults remain to be described. Previous literature on adults has only reported differences between ε2 carriers and non-carriers, or against *ε4* carriers, all showing larger GM volumes or cortical thickness in association with the *ε2* allele, in AD-sensitive regions such as the entorhinal cortex.[13–16] These results suggest that ε2 carriers may have higher brain reserve,[7] which might allow them to better cope with aging and pathology. In line with this, it has been reported that ε2 carriers remain cognitively unimpaired for a longer period even in the rare case of developing AD pathology (*i.e.*, Aβ and tau).[17,18] Studying the brain properties in late-/middle-aged cognitively unimpaired ε2 carriers may increase our understanding of the biological mechanisms associated with this protective allele.

Importantly, to better clarify the mechanisms related to the *APOE* ε2 allele, it would be necessary to test its impact both on brain areas that are the target of incipient degeneration, and on those related to cognitive resilience. The thinning of specific areas such as the entorhinal cortex or temporal areas has shown a tight association with the progression of AD.[19,20] On the other hand, the maintenance of metabolism in other areas, such as the anterior cingulate or the temporal pole, has been related to preserved cognitive function.[21] These facts suggest that metabolic and volumetric measures in these regions are of particular interest when studying characteristics related to AD.

This study aimed to investigate the association between the *APOE* ε2 genotype and brain morphology in late-/middle-aged cognitively unimpaired individuals, with a focus on ε2/ε2 individuals and ε2 allele dose effects. We performed two sets of analyses: a hypothesis-driven analysis in which we studied the ε2 allele effects on areas related to AD (*i.e.*, AD signature and resilience signature); and a hypothesis-free approach in which we expanded these analyses to the whole brain. For both sets of analyses, GM volumes of all *ε2* genotypic groups (*i.e.*, ε2/ε2, ε2/ε3, and ε2/ε4) were compared to the reference *ε3/ε3* group, as well as to one another. The genotypic dose-dependent effects (*i.e.*, dominant, additive, and recessive) of the *ε2* allele were also investigated. Finally, we computed a continuous measure to capture the risk of AD related to the *APOE* genotype (*i.e.*, *APOE* genotype-related AD risk). Effects of this measure on GM volumes were explored and compared to those of the *ε2* allele. We hypothesized that (1) *APOE ε2* carriership would be associated to larger GM volumes in areas known to be affected in AD[19] and areas related to successful aging,[21] (2) a higher dose of *ε2* allele would be related to larger GM volumes, and (3) these effects would contribute to the global *APOE* genotype-related AD risk effect on GM volumes.

## 2 | METHODS

### 2.1 | Participants

Leveraging a previous multi-cohort study,[22] we checked the cohorts for cognitively unimpaired *APOE ε2/ε2* individuals and extended our search to new cohorts. The final selection included: the ALFA (Alzheimer's and Families) study from Barcelona, Spain;[23] the Amsterdam Dementia Cohort (ADC) from the Netherlands;[24,25] the Gothenburg H70 Birth cohort study (H70) from Sweden;[26] the BioFINDER (www.biofinder.se) from Sweden; the Alzheimer's Disease Neuroimaging Initiative (ADNI; http://adni.loni.usc.edu/) from the United States and Canada; and the Open Access Series of Imaging Studies (OASIS; http://www.oasis-brains.org/) from the United States[27] (see supporting information for a description of each cohort). The search in AIBL (Australian Imaging, Biomarker & Lifestyle Study of Ageing; https://aibl.csiro.au/) and in Japanese ADNI (https://humandbs.biosciencedbc.jp/en/hum0043-v1) cohorts did not return any *APOE ε2/ε2* individual in their magnetic resonance imaging (MRI) arms. The search in AddNeuroMed[28] and the CBAS (Czech Brain Aging Study)[29] cohorts

only identified *APOE ε2/ε2* individuals with cognitive impairment, and so were not included in this study.

We first selected all cognitively unimpaired *APOE ε2/ε2* individuals who had T1-weighted MRI data available. The criteria for classifying individuals as cognitively unimpaired were similar in all cohorts, including: normal global cognition as reflected by a Clinical Dementia Rating (CDR) score of 0 or a Mini-Mental State Examination (MMSE) score of 25 or higher, and/or normal cognition as decided by a multidisciplinary consensus panel of experts (see supporting information). After selecting the *APOE ε2/ε2* individuals as the reference group, we selected one participant of each of the other *APOE* genotypes to match every *APOE ε2/ε2* individual using age, sex, and education as matching variables, within each of the cohorts. Because matching was performed within cohorts, the six *APOE* genotype groups were also matched for scanner/protocol except for the ADNI, because the ADNI was designed to provide comparable images across scanners and protocols (http://adni.loni.usc.edu/methods/mri-tool/mri-analysis/). In the ADC, some individuals did not get a match with exactly the same MRI protocol. For those individuals, we selected the most similar MRI protocol in terms of manufacturer, field strength, and acquisition parameters.

## 2.2 | Image processing

Participants were scanned using T1-weighted sequences with comparable scanning protocols and image resolution across cohorts (see the supporting information). GM was segmented and warped into Montreal Neurological Institute (MNI) space following a standard procedure using SPM12 (see supporting information). Images were spatially smoothed with an 8-mm full width at half maximum Gaussian kernel. Total intracranial volume (TIV) was computed as the sum of GM, white matter, and cerebrospinal fluid volume partitions using the CAT12 toolbox.

To calculate regional GM volume we used the cortical and subcortical areas from the Desikan-Killiany[30] atlas. We summed the intensity of the modulated GM images in the MNI space in each region across individuals. We also created two composite regions of interest (ROIs) to specifically investigate the brain areas known to be typically affected in AD, as well as areas known to be associated with successful aging or resilience. Following previous studies, the AD signature ROI was created by combining the entorhinal cortex, inferior and middle temporal and fusiform gyrus[19]; and the resilience signature ROI was created by combining the anterior cingulate and temporal pole regions.[21] We also performed asymmetry analysis (see the next section), which included a medial-temporal lobe (MTL) composite ROI that combined hippocampus, amygdala, and parahippocampal ROIs.[16]

## 2.3 | Statistical analysis

We compared the demographic characteristics across *APOE* genotypes using analysis of variance (for continuous variables) and $\chi^2$ (for categorical variables). All analyses described below for *APOE* effects on GM volume were performed in two different sets of analyses. First, we specifically tested for *APOE* effects on areas related to AD (*i.e.*, AD signature and resilience signature). Second, we expanded the approach to a whole-brain analysis.

### RESEARCH IN CONTEXT

1. **Systematic review**: The authors reviewed previous literature related to apolipoprotein E (*APOE*) effects on Alzheimer's disease (AD)-related phenotypes, with specific attention to those linked to brain morphology. Although studies focusing on the effects of the *APOE ε2* allele, and especially of the *ε2* homozygosity, are scarce, we have cited those related to our research.
2. **Interpretation**: Our study increases our knowledge about *APOE*, and especially *APOE ε2*, effects on gray matter volumes and suggest a mechanism through which *APOE ε2* homozygotes may maintain their cognitive function throughout life even in the improbable case of developing AD pathology.
3. **Future directions**: In this study, we have proved the value of studying gene dose effects of the *ε2* allele over the frequently investigated carriership effects. Future studies about the *APOE ε2* allele need to further explore these gene dose effects on other important AD-related phenotypes. This may help us to understand the exceptional low risk of these subjects to develop AD dementia.

### HIGHLIGHTS

- Cognitively unimpaired *ε2* carriers have larger gray matter (GM) volumes than *ε3* homozygotes.
- Apolipoprotein E (*APOE*) *ε2* carriership is associated with larger GM volumes in the Alzheimer's disease signature.
- *APOE ε2* homozygotes have larger GM volumes in areas related to cognitive resilience.
- Genotypic *APOE* effect on GM volume is not only due to *ε4* but also to *ε2* effects.

### 2.3.1 | Comparisons between *APOE ε2* genotypic groups

We first compared each *ε2* genotypic group (*i.e.*, *ε2/ε2*, *ε2/ε3*, and *ε2/ε4*) to the reference group (*i.e.*, *ε3* homozygotes), and also to each other (*i.e.*, *ε2/ε2* vs. *ε2/ε3*, *ε2/ε2* vs. *ε2/ε4*, and *ε2/ε3* vs. *ε2/ε4*). Generalized linear models were used to compare each pair of groups to GM volume as the dependent variable and the *APOE* genotype as the variable of

interest. Age, sex, education, scanner (as a dummy variable), and TIV were included as covariates. The models used were analogous for both sets of analyses (*i.e.*, AD composites and whole brain).

#### 2.3.2 | Dose-dependent effects of the *APOE ε2* allele on GM volume

The second aim of this study was to investigate particular dose-dependent effects of the *ε2* allele on GM volumes. Similar statistical models were used in these analyses but including only *ε2* carriers and *ε3* homozygotes in this case. *APOE ε2/ε4* participants were excluded from this analysis to avoid the influence of the *APOE ε4* allele.[31] Contrasts were designed to test for dominant (*i.e.*, *APOE ε2* carriers vs. *APOE ε3/ε3* individuals), additive (*i.e.*, *APOE ε2/ε2* vs. *APOE ε2/ε3* vs. *APOE ε3/ε3*), and recessive (*i.e.*, *APOE ε2/ε2* vs. *APOE ε2/ε3* plus *APOE ε3/ε3*) effects of the *APOE ε2* allele.[32]

As an additional analysis we also investigated right-left hemispheric asymmetry[16,33] on GM volume in the MTL as a composite and each of the ROIs included in the MTL composite (*i.e.*, hippocampus, amygdala, and parahippocampus). We replicated the previous analysis using the asymmetry metrics as dependent variables and the same covariates excluding TIV, as the asymmetry value is already normalized by the total volume of the region itself.

#### 2.3.3 | Comparison of *ε2* and global *APOE* genotype-related AD risk effects on GM volume

The third aim of this study was to investigate the *APOE* genotypic effect on GM volume and compare it to the previous *ε2* dose-dependent effects. We created a new variable that we called "*APOE* genotype-related AD risk," which encoded the risk of AD for each of the genotypes as a continuous variable. Our goal was to create a measure related to the *APOE* genotype that would capture the related risk of developing AD and investigate whether this was associated to GM volumes. This variable was calculated by log-transforming previously published odds ratios for developing AD associated to each *APOE* genotype, with *APOE ε3/ε3* individuals as the reference group (Table S1 in supporting information).[3] We repeated the composite-based and the whole-brain analyses using the *APOE* genotype-related AD risk value as an independent variable (as a continuous variable). In addition, we also performed Spearman's rank correlations between the regional effects of the *APOE* genotype-related AD risk and the dose-dependent effects of the *ε2* allele. These correlations aimed to compare global *APOE* and *ε2* standalone effects on GM volumes.

For all three objectives, statistical significance was set at $P < 0.05$ using the false discovery rate (FDR) adjustment for multiple testing (hypothesis-free approach), and at uncorrected $P < 0.05$ for the a priori selected areas related to AD (hypothesis-driven approach). In addition, supporting information shows the results from uncorrected $P < 0.05$ in the hypothesis-free approach, for completeness of information.

## 3 | RESULTS

### 3.1 | Participants

The sample was composed of 223 cognitively unimpaired individuals, including 38 *APOE ε2/ε2* individuals and 38 matched individuals for each of the other *APOE* genotypes (except for the *APOE ε2/ε4* group, which included 33 participants due to unavailability of suitable matches for 5 cases). All individuals were matched for age, sex, and education within the center. As shown in Table 1, there were no statistically significant differences in these variables by the *APOE* group. Moreover, MMSE scores and TIV did not show significant differences among *APOE* groups.

### 3.2 | Comparisons between *APOE ε2* genotypic groups

We first investigated whether there were significant differences between groups on two AD-related GM volume ROI composites: the AD signature and the resilience signature. *APOE ε2/ε3* participants had larger GM volume in the AD signature areas compared to *ε3* homozygotes (Table 2 and Figure 1). Within the resilience signature, *ε2* homozygotes had larger GM volumes than the *ε3/ε3* and *ε4/ε4* groups.

In the whole-brain analysis, *ε2/ε2* and *ε2/ε3 APOE* groups showed larger GM volumes than *ε3* homozygotes (Figure 2A). Differences between *ε2/ε2* and *ε3* homozygotes and between *ε2/ε3* and *ε3* homozygotes were widespread across the brain. On the other hand, *ε2/ε4* participants only showed larger volumes in the inferior parietal and in the inferior temporal gyri, although these differences did not survive the FDR adjustment (Figure S1A in supporting information).

When studying differences between *ε2* genotypic groups, we found the largest differences between *ε2/ε2* and *ε2/ε4* groups, including bilateral postcentral gyri, and right parahippocampal and posterior cingulate gyri (Figure 2B). *APOE ε2* homozygotes only showed larger volumes than *ε2/ε3* participants in the right precentral gyrus, but this difference did not survive the FDR adjustment (Figure S1B). In addition, *ε2/ε3* had larger volumes than *ε2/ε4* participants in bilateral postcentral gyri.

### 3.3 | Dose-dependent effects of the *APOE ε2* allele on GM volume

In the ROI analyses, we found a significant dominant effect of higher GM volume associated with the *ε2* allele on both AD-related composites (Table 2). The additive effect also showed a trend to significance in the same direction for the resilience signature, but no other significant effects were observed.

Figure 3 shows the significant areas that had a positive association between GM volume and each of the gene-dose effects of the *ε2* allele in whole-brain analyses, after the FDR adjustment for multiple testing. Uncorrected results can be found in the supporting information

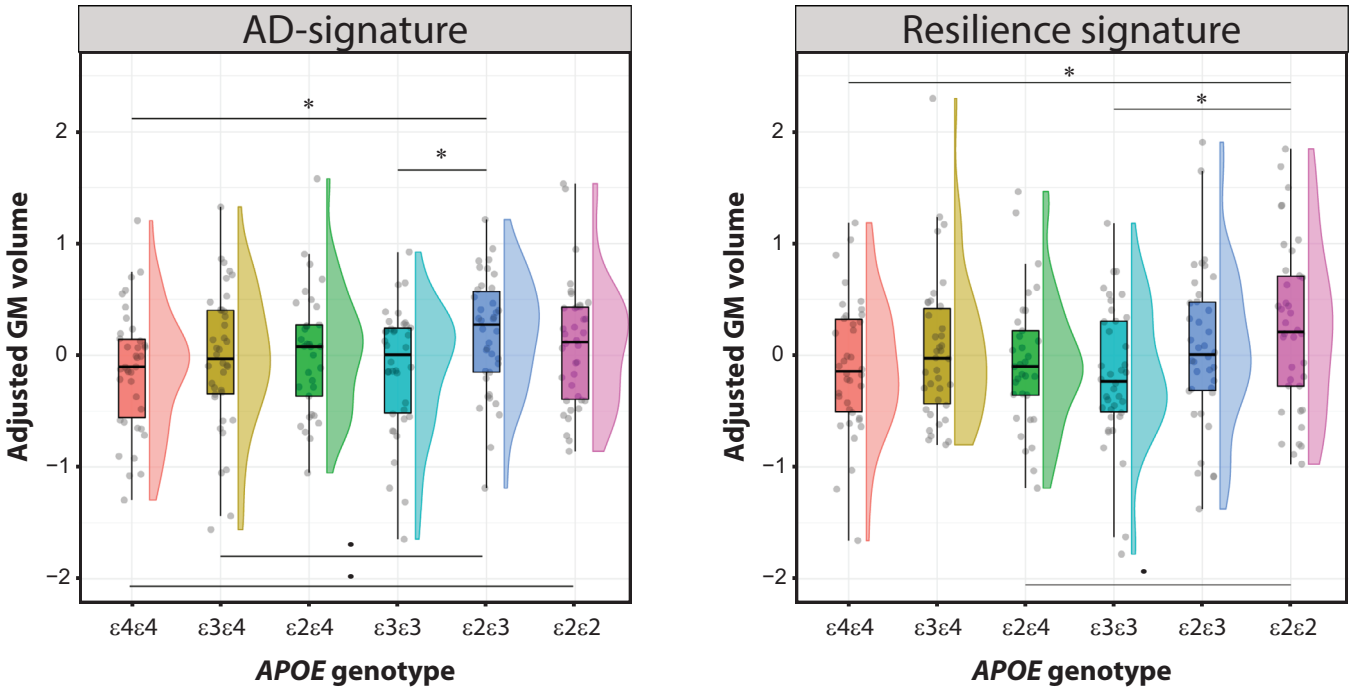


**FIGURE 1** Association between *APOE* genotype and GM volume in AD-related areas. Adjusted GM volume in areas affected in AD (AD signature;[19] left) and in areas known to be associated with successful aging or resilience (resilience signature; right)[21] by *APOE* genotype. GM volumes were adjusted by age, sex, education, scan, and TIV. $^*P < 0.05$; $\cdot\ P < 0.10$. AD, Alzheimer's disease; APOE, apolipoprotein E; GM, gray matter; TIV, total intracranial volume

for completeness of information (Figure S2). The dominant effect was the most widespread including multiple AD-related areas such as the fusiform gyrus, precuneus, or the posterior cingulate. Additive effect was also widespread, although less so than the dominant effect. Finally, the recessive effect was only significant in the paracentral and the pars opercularis of the right hemisphere. Negative associations were not observed for any of the three effects (*i.e.*, the *ε2* allele was not associated with a smaller GM volume in any brain region).

Additional analyses for asymmetry effects in the subregions of MTL showed that the *ε2* recessive, dominant, and additive effects were stronger in the right hemisphere only in the parahippocampal gyrus (Figure S3 in supporting information). More specifically, *ε2* homozygotes had greater right–left asymmetry (R > L) than *ε3* homozygotes.

### 3.4 | Comparison of *ε2* and global *APOE* genotype-related AD risk effects on GM volume

In the composite-based analysis, the *APOE* genotype-related AD risk showed a negative association with GM volume in the AD signature ($F = 4.61$, $P = 0.033$) and a trend in the resilience signature, in the same direction ($F = 2.81$, $P = 0.096$). Both results indicate lower GM volumes for a higher risk of AD, which is related to the *APOE* genotype in these areas (Table 2). Figure 4A shows the specific regions of this negative correlation for the *APOE* genotype-related AD risk with GM volume. In particular, the increased risk of AD dementia related to *APOE* genotype correlated with less GM volume in brain areas overlapping with parts of the AD signature such as the entorhinal and the fusiform, as well as with parts of the resilience signature such as the anterior cingulate. Results uncorrected for multiple comparisons can be found in Figure S4 in supporting information.

We then compared these results to those ones from dose-dependent effect of the *ε2* allele, to investigate whether the GM volume effects related to the risk of AD are only due to the *ε4* allele or may also be due to the *ε2* allele. We found significant negative correlations for the dominant *ε2* allele effect ($\rho = -0.35$, $P = 0.004$) and the additive *ε2* effect ($\rho = -0.27$, $P = 0.028$), indicating opposite effects of the *ε2* allele (*i.e.*, protective) and the *APOE* genotype-related AD risk (*i.e.*, deleterious). No significant correlations were observed for the recessive *ε2* effect ($\rho = -0.13$, $P = 0.275$, Figure 4B).

## 4 | DISCUSSION

In this multi-cohort study, we investigated genotypic and dose-dependent effects of the *ε2* allele on GM volumes in late-/middle-aged cognitively unimpaired individuals. As hypothesized, we found that the *ε2* allele was associated with larger GM volumes in brain areas relevant for AD. However, the dose-dependent effect of this allele was distinct for different areas. Regions typically affected by AD-related neurodegeneration were similarly protected by the carriership of at least one *ε2* allele, regardless of their load. On the other hand, areas related with cognitive maintenance presented larger volumes in relation with the dose (*i.e.*, number) of this allele. In particular, *APOE ε2* homozygotes, but

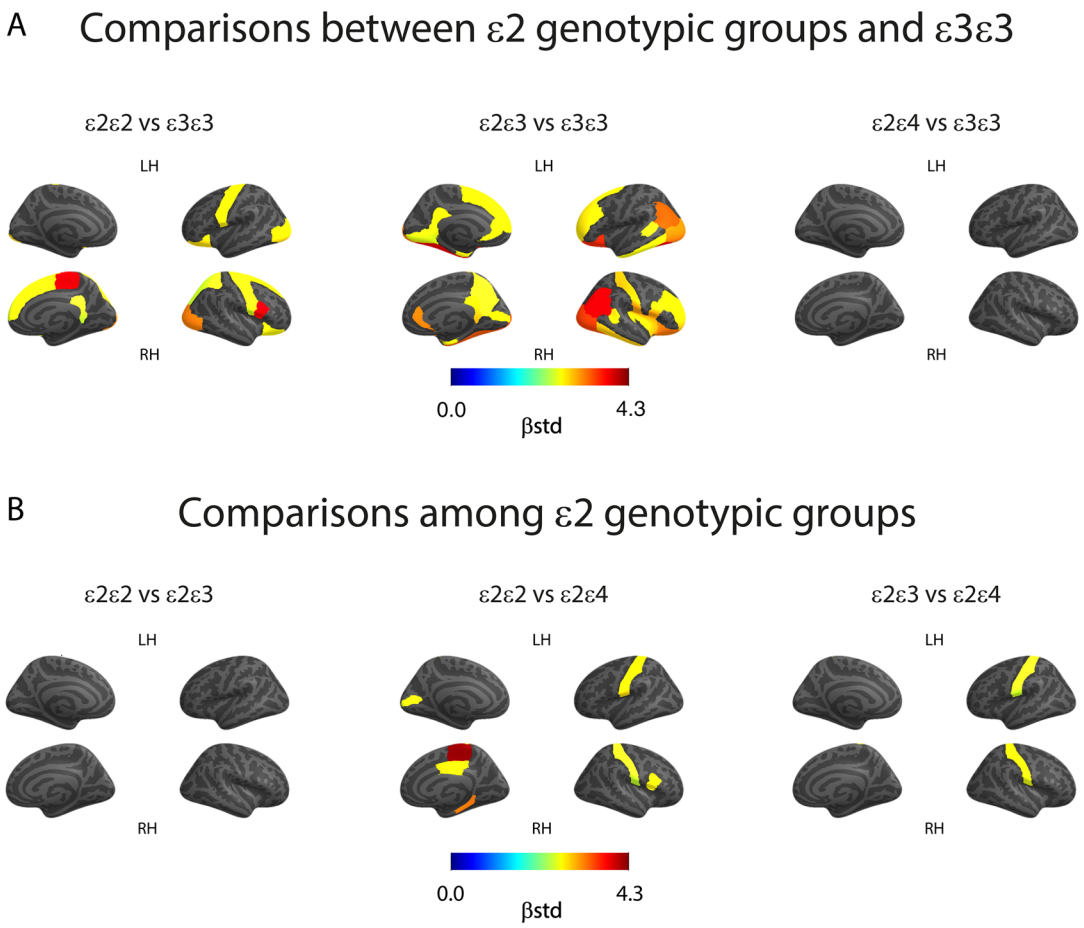


**FIGURE 2** Comparisons between *APOE ε2* genotypic groups. Comparisons between *APOE ε2* genotypic groups and *APOE-ε3* homozygotes as the reference group (A); and between each pair of *APOE ε2* genotypic groups (B). Colors indicate the effect size of each effect in regions that were statistically significant ($P < 0.05$ FDR-adjusted). AD, Alzheimer's disease; *APOE*, apolipoprotein E; FDR, false discovery rate; GM, gray matter; LH, left hemisphere; RH, right hemisphere

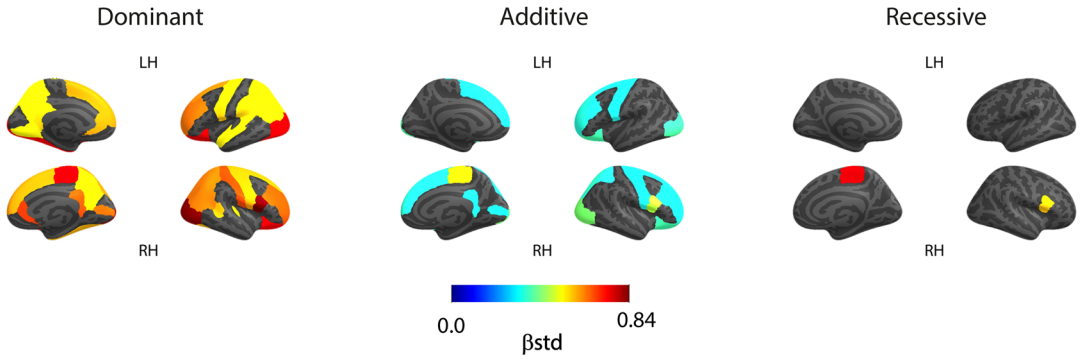


**FIGURE 3** Dose-dependent effects of the *APOE ε2* allele. Dose-dependent effects of the *ε2* allele on GM volume (from left to right: dominant, additive, and recessive). *APOE ε2/ε4* participants were not included in this analysis. Colors indicate the effect size of each effect in regions that were statistically significant ($P < 0.05$ FDR-adjusted). AD, Alzheimer's disease; *APOE*, apolipoprotein E; FDR, false discovery rate; GM, gray matter; LH, left hemisphere; RH, right hemisphere

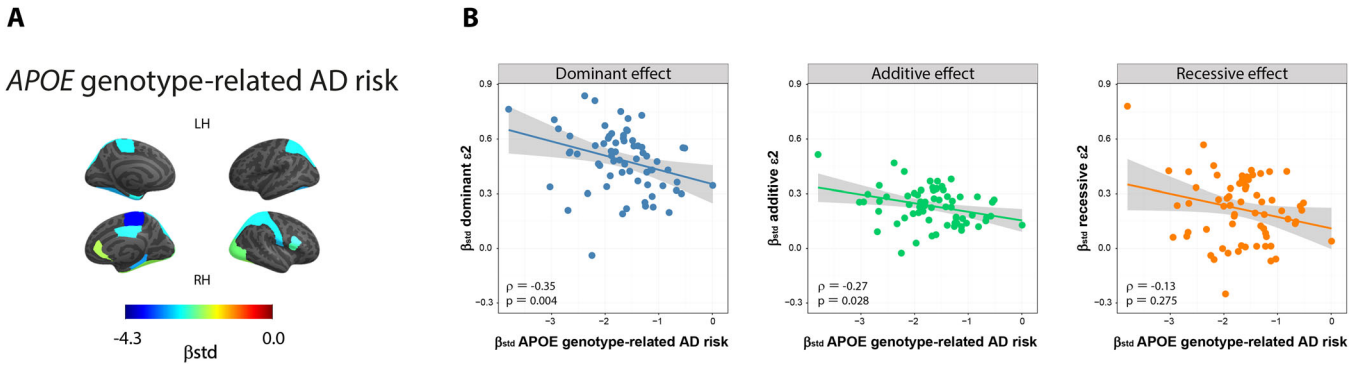


**FIGURE 4** *APOE* genotype-related AD risk effect on GM volume and association to *APOE ε2* effects. *APOE* genotype-related AD risk effects on GM volume (A). Colors indicate the effect size of each effect in regions that were statistically significant ($P < 0.05$ FDR-adjusted). Associations between dose-dependent effects of the *ε2* allele ($\beta_{std}$) on GM volume (from left to right: dominant, additive, and recessive) and *APOE* genotype-related AD risk effect ($\beta_{std}$) on GM volume. Spearman's $\rho$ and *P*-values are shown in the left bottom corner of each plot. AD, Alzheimer's disease; *APOE*, apolipoprotein E; FDR, false discovery rate; GM, gray matter; LH, left hemisphere; RH, right hemisphere

not *APOE ε2* heterozygotes, showed larger GM volumes than *APOE ε3* homozygotes in the resilience signature. This was further supported by the dose-dependent effects analyses, in which we found a trend to significance in the additive model for the *ε2* allele, in the resilience but not in the AD signature. Finally, the effect of this protective allele seemed to be spatially related to that of an AD risk measure including all *APOE* genotypes, suggesting that the *ε2* allele plays an opposing effect to that of the *ε4* allele on GM volumes.

Our findings extend previous studies showing that having at least one *ε2* allele confers larger GM volumes in areas known to be affected in AD.[13–15] For instance, the effect of the *ε2* carriership on entorhinal volume has previously been observed in cognitively unimpaired individuals,[13,15] as well as in patients with mild cognitive impairment (MCI) and AD dementia.[34] A stepwise difference (ε2 carriers > ε3/ε3 individuals > ε4 carriers) in cortical thickness in the entorhinal cortex was also found in children and adolescents.[35] Further, our results together with a previous study with A*β*-positive subjects suggest that this protective effect of the ε2 allele in the MTL, and more specifically in the parahippocampus, may be more pronounced in the right hemisphere.[16]

In our study we also expand previous findings to regions related with cognitive resilience.[21,36] This result suggests that the well-known low risk of cognitive decline in *ε2* carriers may not only be due to a low risk of accumulating A*β*[4] and tau[37] pathologies (*i.e.*, resistance to AD pathology), but also to preserved brain integrity in areas that are associated with greater resilience to, or capacity to cope with AD pathology.[21] A contribution of our study is that we demonstrated that the protective effect on these areas was particularly related to the homozygosity of the *ε2* allele. Greater GM volume in areas related to cognitive resilience may also explain why the oldest old *ε2* carriers could remain clinically non-demented even when displaying elevated AD pathology.[17,18] With these results in mind, we propose that the increased brain reserve found in *ε2* carriers, and especially in *ε2* homozygotes, may promote their maintained cognitive functions, even in the rare case of developing AD pathology.

The unique design of our study allowed us to study the *ε2* allele effects on GM volume in more detailed ways than previous studies. More specifically, we found that carrying one *ε2* allele always seemed to confer an advantage compared to *ε3* homozygotes, even when an *ε4* allele is also present, although this last result did not survive the adjustment for multiple comparisons. Moreover, our results suggest that having an extra copy of the *ε2* allele did not translate into a major gain in areas usually related to neurodegeneration, but it did on resilience areas. Thus, suggesting that carrying one *ε2* allele may be sufficient to prevent or decrease AD-related neurodegeneration, maybe in part through lowering AD pathology levels. However, adding an extra copy of the *ε2* allele may not be beneficial for GM integrity in these areas, which may be related to the increased risk of *APOE ε2* carriers to having cerebrovascular problems.[16] On the other hand, being *ε2* homozygote increased brain reserve in areas related to cognitive resilience, which may in turn delay their cognitive decline and explain their higher survival rate without AD dementia.[3] Altogether, our results highlight that comparing all *ε2* genotypic groups is superior to merging all *ε2* carriers, when it comes to disentangling the specific effect of the *ε2* allele and advance our understanding of its protective effects.[3] This accomplishment was an advantage of our large multi-cohort design that has not been possible in previous single-center studies.

Finally, we investigated the effect of a measure capturing the risk of AD due to the *APOE* genotype (*i.e.*, *APOE* genotype-related AD risk) on GM volumes and compared it to dose-dependent effects of the *ε2* allele. As hypothesized, higher *APOE* genotype-related AD risk conferred smaller GM volumes both in areas targeted by AD pathology and areas related with brain resilience.[19–21] This finding is important and extends previous reports on the *APOE ε4* allele[5,38,39] to now also incorporate the effect of the *APOE ε2* allele. Our results suggest that carrying at least one *ε2* allele contributed to this effect. The reason the recessive effects of the *ε2* allele are not associated with those of the *APOE* genotype-related AD risk may be related to its corresponding upstream mechanisms. While the *APOE* genotype-related AD risk may show more important effects on AD-neurodegeneration

areas,[19] which may be partially due to Aβ and tau deposition effects, *ε2* homozygotes showed a more pronounced effect on areas of cognitive resilience not typically associated with pathology, which may come from developmental characteristics.

The potential underlying mechanisms for the larger GM volumes in *APOE ε2* carriers are unknown and they deserve further research. However, previous studies suggested that these mechanisms may involve both Aβ-dependent and -independent pathways.[11] *APOE ε2* carriers produce the APOE *ε2* isoform, which is not only beneficial in terms of Aβ production, aggregation, and clearance,[40,41] but also protects against Aβ toxicity through a reduction of its oligomerization.[42] Therefore, by avoiding Aβ pathology, late-/middle-aged *APOE ε2* homozygotes may be relatively spared of Aβ-related effects on GM volume, particularly compared to *ε4* carriers. In addition, the APOE *ε2* isoform has also shown Aβ-independent effects that may explain our current results. For instance, the APOE *ε2* isoform promotes synaptic integrity, facilitates anti-oxidant and anti-inflammatory activity, and may mediate neuronal growth through a more efficient lipid and cholesterol metabolism (see Liu et al.,[2] Li et al.,[11] Suri et al.,[40] and Yamazaki et al.[41] for detailed reviews). Altogether, these mechanisms may induce better neuronal health throughout the full lifespan and may partially explain the observed larger GM volumes in *APOE ε2* carriers.

The multi-cohort nature of our study is not free from limitations. First, not all participants in this sample had biomarkers of AD pathology available, which prevented us from investigating whether lower GM volume in the AD signature is in fact related to AD pathology. However, the extremely low prevalence of Aβ pathology in cognitively unimpaired *APOE ε2* carriers suggests that the impact of this limitation is minor on the observed *ε2* effects, which constitute the main focus of our work.[3,43] Second, to maximize the number of *APOE ε2/ε2* individuals we pooled MRI data from different scanners, which may have introduced bias related to scanner-specific features such as geometric distortion and tissue contrast. We minimized this issue by a strict control of several factors, at two levels. At the study design level, we strictly matched the six *APOE* genotype groups in terms of MRI scanner/protocol, in addition to our matching for age, sex, and years of education. In addition to this control at the design level, we conducted another correction at the statistical level, to remove the potential residual confounding effect of all these variables, including the MRI scanner/protocol. Therefore, these variables are unlikely to affect our current results in a significant manner. Third, the criterion for "normal cognition" was similar but varied slightly across cohorts. Nonetheless, all the cohorts used criteria commonly applied in clinical routine and in aging research.

In conclusion, in late-/middle-aged cognitively unimpaired individuals, the *APOE ε2* allele is associated with larger GM volume in AD-related brain regions. However, distinct dose-dependent effects of this allele were observed in different areas of the brain. It is important to note that *APOE ε2* homozygotes had a specific protective effect in areas related to cognitive resilience. Furthermore, *APOE ε2* effects on GM volumes were similar, but opposed, to those related to the *APOE ε4* genotypes. Altogether, our large multi-cohort data advocates for increased brain reserve in *APOE ε2* carriers, especially in *ε2*

**TABLE 1** Sample characteristics

| | All (n = 223) | ε2/ε2 (n = 38) | ε2/ε3 (n = 38) | ε3/ε3 (n = 38) | ε2/ε4 (n = 33) | ε3/ε4 (n = 38) | ε4/ε4 (n = 38) | *P*-value |
|---|---|---|---|---|---|---|---|---|
| Age (years old), mean (SD) [min–max] | 64.6 (8.9) [45.8–88.0] | 65.0 (9.1) [49.8–88.0] | 64.9 (8.8) [47.9–87.0] | 65.0 (9.2) [48.8–88.0] | 64.2 (9.2) [45.8–88.0] | 64.7 (9.2) [50.5–88.0] | 64.0 (8.7) [52.1–81.0] | 0.994 |
| Women, n (%) | 99 (44.4) | 17 (44.7) | 16 (42.1) | 17 (44.7) | 14 (42.7) | 18 (47.4) | 17 (44.7) | 0.998 |
| Education (years), mean (SD) | 14.2 (3.6) | 14.2 (3.8) | 14.3 (3.6) | 14.5 (3.7) | 13.8 (3.2) | 14.1 (3.7) | 14.1 (3.4) | 0.973 |
| MMSE, mean (SD)* | 28.9 (1.2) | 29.2 (1.0) | 29.0 (1.1) | 29.1 (1.0) | 28.8 (1.2) | 28.6 (1.8) | 28.8 (1.0) | 0.417 |
| TIV ($cm^3$), mean (SD) | 1491.4 (146.7) | 1481.3 (1287.1) | 1474.4 (126.7) | 1486.6 (142.9) | 1505.8 (158.9) | 1490.3 (130.0) | 1512.1 (133.7) | 0.878 |
| Cohort: ADC/ADNI/ALFA/BioFINDER/H70/( | 72/18/42/31/18/42 | 12/3/7/6/3/7 | 12/3/7/6/3/7 | 12/3/7/6/3/7 | 12/3/7/1/3/7 | 12/3/7/6/3/7 | 12/3/7/6/3/7 | - |

*MMSE from one individual is missing.

Abbreviations: ADC, Amsterdam Dementia Cohort; ADNI, Alzheimer's Disease Neuroimaging Initiative; ALFA, Alzheimer's and Families; H70, Gothenburg H70 Birth cohort study; MMSE, Mini-Mental State Examination; OASIS, Open Access Series of Imaging Studies; SD, standard deviation; TIV, total intracranial volume.

**TABLE 2** *APOE* effects on GM volumes in AD-related areas

| | AD signature | | Resilience signature | |
|---|---|---|---|---|
| | $\beta_{std}$ [95%CI] | *P*-value | $\beta_{std}$ [95%CI] | *P*-value |
| **Comparisons between *ε2* genotypic groups** | | | | |
| *ε2/ε2* vs. *ε3/ε3* | 1.41 [−0.56, 3.38] | 0.161 | **2.36 [0.39, 4.33]** | **0.019** |
| *ε2/ε3* vs. *ε3/ε3* | **2.71 [0.74, 4.69]** | **0.007** | 1.66 [−0.33, 3.63] | 0.101 |
| *ε2/ε4* vs. *ε3/ε3* | 0.95 [−1.02, 2.92] | 0.342 | 0.62 [−1.36, 2.59] | 0.540 |
| *ε2/ε2* vs. *ε2/ε3* | −1.22 [−3.19, 0.74] | 0.222 | 0.75 [−1.22, 3.65] | 0.453 |
| *ε2/ε2* vs. *ε2/ε4* | 0.41 [−1.56, 2.38] | 0.680 | *1.67 [−0.31, 3.64]* | *0.098* |
| *ε2/ε3* vs. *ε2/ε4* | 1.61 [−0.36, 3.58] | 0.109 | 0.95 [−1.03, 2.93] | 0.347 |
| **Dose-dependent effects** | | | | |
| Dominant *ε2* | **2.64 [0.66, 4.62]** | **0.010** | **2.07 [0.09, 4.05]** | **0.041** |
| Additive *ε2* | 1.60 [−0.39, 3.58] | 0.114 | 1.92 [−0.07, 3.87] | *0.058* |
| Recessive *ε2* | 0.09 [−1.88, 2.07] | 0.926 | 0.21 [−0.02, 0.47] | 0.239 |
| ***APOE* genotype-related AD risk** | **−2.15 [−4.11, −0.17]** | **0.033** | *−1.67 [−3.63, 0.30]* | *0.096* |

Notes: Results of the analysis of the comparisons between *ε2* genotypic groups; dose-dependent (additive, recessive, and dominant) effects of the *ε2* allele and *APOE* genotype-related AD risk effect on GM volume in areas related to AD.[19,21] The first column of each effect shows the $\beta_{std}$ (calculated as the estimate divided by SE) and 95%CI, the second the respective *P*-value. A negative value of the last row shows a negative correlation between GM volume and the *APOE* genotype-related AD risk, meaning more GM volume for a lower AD risk related to *APOE* genotype. Significant results ($P < 0.05$) are shown in bold and those that showed a trend to significance ($P < 0.100$) are shown in italics

Abbreviations: AD, Alzheimer's disease; *APOE*, apolipoprotein E; CI, confidence interval; GM, gray matter; ROI, region of interest; SE, standard error; *β*std, standardized *β*.

homozygotes, which may in turn confer them additional protection against AD-related cognitive decline, independent of the well-known effects of *APOE* on A*β*.

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## CONFLICTS OF INTEREST

JLM is currently a full-time employee of Lundbeck and priorly has served as a consultant or at advisory boards for the following for-profit companies, or has given lectures in symposia sponsored by the following for-profit companies: Roche Diagnostics, Genentech, Novartis, Lundbeck, Oryzon, Biogen, Lilly, Janssen, Green Valley, MSD, Eisai, Alector, BioCross, GE Healthcare, ProMIS Neurosciences. HZ has served on scientific advisory boards for Alector, Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics, and CogRx; has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, and Biogen; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). KB has served as a consultant, on advisory boards, or on data monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu. Julius Clinical, Lilly, MagQu, Novartis, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. GK is a full-time employee of Roche Diagnostics GmbH. IS is a full-time employee and shareholder of Roche Diagnostics International Ltd. The remaining authors declare that they have no conflicts of interest.

## AUTHOR CONTRIBUTIONS

Gemma Salvadó and Daniel Ferreira contributed to the design and implementation of the research, to the analysis of the results, and to the writing of the manuscript. Grégory Operto, Irene Cumplido-Mayoral, Raffaele Cacciaglia, Carles Falcon, and Colin Groot contributed to analysis of the results. Eider M. Arenaza-Urquijo, Natàlia Vilor-Tejedor, Rik Ossenkoppele, and José Luis Molinuevo aided in interpreting the results and worked on the manuscript. Wiesje M. van der Flier, Frederik Barkhof, Philip Scheltens, Rik Ossenkoppele, Silke Kern, Anna Zettergren, Ingmar Skoog, Jakub Hort, Erik Stomrud, Danielle van Westen, Oskar Hansson, José Luis Molinuevo, Lars-Olof Wahlund, Eric Westman, and Juan Domingo Gispert contributed to sample preparation. Eric Westman and Juan Domingo Gispert contributed to the design and implementation of the research. All authors discussed the results and commented on the manuscript.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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