SEX-SPECIFIC PROTECTIVE EFFECTS OF *APOE* ε2 ON COGNITIVE PERFORMANCE

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Abstract

Apolipoprotein E (*APOE*) has an important role in the multiple trajectories of cognitive aging. However, environmental variables and other genes mediate the impact of *APOE* on cognition. Our main objective was to analyze the effect of *APOE* genotype on cognition and its interactions and relationships with sex, age, lipid profile, C-reactive protein (CRP) and *Brain derived neurotrophic factor (BDNF*) genotype in a sample of 648 healthy subjects over 50 years of age with a comprehensive neuropsychological assessment. Our results showed that *APOE ε2* carriers performed better in the Verbal Memory *(p* = 0.002) and Fluency Domains *(p* = 0.001). When we studied the effect of sex, we observed that the beneficial effect of *APOE ε2* on the normalized values of these cognitive domains occurred only in females (β = 0.735; 95% *CI*, 0.396-1.074; $p = 3.167 \cdot 10^{-5}$ and β = 0.568; 95% *CI*, 0.276-0.861; $p = 1.853 \cdot 10^{-4}$, respectively). Similarly, the sex-specific effects of *APOE ε2* were further observed on lipidic and inflammation biomarkers. In the whole sample, *APOE ε2* carriers showed significantly lower levels of total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C) and CRP. These differences were found only among females. Furthermore, TC and LDL-C mediated the protective effect of *APOE ε2* on cognition in the whole sample and TC in females, providing candidate physiological mechanisms for the observed genetic effects. Our results show that the neuroprotective role of *APOE ε2* in cognition varies with sex and that the lipidic profile partially mediates this protection.

Keywords: *APOE, BDNF*, sex differences, cognition, lipid profile, C-reactive protein

INTRODUCTION

Age-related cognitive and functional decline is a continuous biological process with different cognitive trajectories [1]. Complex interactions between heritability, environmental influence, and cognitive functions in aging have been highlighted [2]. In particular, genetic differences explain around 15–25% of the variance in life expectancy [3]. Therefore, the identification of susceptibility genes and their biological effects on cognitive aging is required to establish inter-individual differences in this process and promote early personalized interventions to delay cognitive decline and minimize the financial burden of aging in the healthcare system.

Apolipoprotein *E* (*APOE*) is one of the primary susceptibility genes consistently related with cognitive loss, dementia, and longevity [4]. This gene has three main allelic variants (*ε2, ε3* and *ε4*). The *ε3* variant is the most frequent allele, present between 60-90%, whereas ε2 and ε4 are present with lower frequencies, 0-20% and 10-20%, respectively [4]. *APOE* is involved in lipid and amyloid-β metabolism, mitochondrial function[5], spine density and dendritic complexity [6], inflammation and neural repair[7]. The functional implications of the different allelic variants have been traditionally interpreted from a deleterious-protective perspective across the lifespan. The ε4 allele has been mainly studied for its association as a genetic risk factor for late-onset Alzheimer's disease (LOAD) [8], cardiovascular disease, stroke, cognitive loss in healthy, mild cognitive impairment (MCI) and Alzheimer's disease (AD) subjects [9]. It has also been associated with structural and functional brain changes in demented and healthy populations [8]. Conversely, the ε2 allelic variant has been positively related to cognitive performance in different cognitive functions (e.g., general cognitive function, episodic memory, executive and language function) during aging [10]. It has also been related to neuroprotective effects against small vessel disease [11] and AD [12] by increasing neural plasticity and synaptic replacement [13], and reduced age-

associated cognitive decline in healthy and demented populations [14,15]. This allelic variant has attracted, however, less attention in comparison with ε4. This situation might be due either to its low frequency, to the main weight conferred to ε4 in cognitive decline and dementia or to the fact that research has either excluded ε2 carriers or pooled them together with *ε3* in a non-ε4 group.

Recently, this deleterious-protective dichotomy between the different allelic variants has been challenged by other studies. In the case of the ε4 allelic variant, there are different studies in which the deleterious effect of ε4 on cognition has not been found [16], or positive effects have been stated [17]. In the case of ε2, it has been shown that ε2 carriers have deleterious effects during early periods of life [17]. All those different studies reveal that *APOE* genotypes may impact differently on cognition during different life stages (Tuminello, E.R. & Han, 2011). Therefore, the influence of *APOE* should be considered within a continuum in which there is not a clear threshold between their protective or deleterious effects [13].

Potential factors that may interact with the effect of *APOE* on cognition are sex, lipidic profile, inflammation, and interactions with other genes. Regarding sex, different studies have pointed out its modulating effect in the relation between *APOE* ε4, cognition and risk of AD [4,19]. These studies and others have highlighted a greater detrimental impact of the ε4 allele in women for different AD biomarkers, such as cerebrospinal fluid (CSF) and brain tau deposition [20], amygdala and hippocampi volumes [19] and increased general AD risk [21]. However, existing literature focused on the interactive effects of sex**APOE* ε4 on cognitive performance in community-dwelling older adults is scarce [22].

Regarding lipidic profile, *APOE* is the major cholesterol carrier in the brain, and it is involved in lipid metabolism and transport [23]. *APOE* genotypes explain 2-5% of the variation in

total cholesterol (TC) plasma levels [24] and modify low-density lipoprotein cholesterol (LCL-C) levels, being LDL-C levels approximately 30% lower in *ε2/ε2* than in *ε4/ε4* individuals [25].

A process tightly linked to the metabolism of lipids in the development of cognitive impairment at older ages is inflammation [26]. Among others, C-reactive protein (CRP) is an important regulator and a sensitive marker of inflammatory processes [27]. *APOE* is also involved in inflammation being *APOE ε4* associated with higher levels of inflammation but lower CRP levels, possibly due to a down-regulation of the mevalonate/cholesterol synthetic pathway [28], reinforcing the links between lipidic profile and inflammation.

Genes do not act in isolation and the inclusion of gene-gene interactions contribute to explain the observed heterogeneity in the effect of the different *APOE* [29] alleles. Among other genes, the *Brain derived neurotrophic factor (BDNF)* has a central role in cognition. *BDNF* is involved in synaptic plasticity, neuronal growth [30], and long-term potentiation related to memory [31]. In general, it has been established that the *Val66Met* polymorphism is related to the amount of BDNF produced by the neurons and, successively, to cognitive function [32]. Nevertheless, there is no consensus about the different effects of the *BDNF* alleles in cognition. Whereas some studies found that the Met allele was associated with declining in multiple cognitive domains compared to Val homozygotes [33], other studies did not report that association [34] or even found the opposite effect on cognition [35]. One possible explanation for the different results might be the interactive effect of the Val66Met polymorphism with sex [36], and *APOE*. Among genetic interactions, it has been reported that the combination *APOE ε*4+ and *BDNF* Met allele is significantly associated with worse memory performance compared to other genotypes [37]. Most consistent results suggest that *APOE ε*2 and *ε*3 are positive regulators of *BDNF* having a possible protective effect. At the

same time ε4 carriers produce less mature BDNF and are therefore at a higher risk of developing AD [38].

Our main objective is to analyze the effect of genetic variants in the *APOE* and *BDNF* genes in cognition and their interactions in a healthy middle-aged population-based sample from the Barcelona AsIA- Neuropsychology Project (AsIA-NP). We hypothesized that *APOE* ε4 and *BDNF* Met allele carriers will perform worse in cognition compared to *APOE* ε2 carriers, while *APOE* ε2 carriers will show a protective effect. These results would be modified by age, sex, and gene-gene interactions. Secondarily, we will analyze the effect of *APOE* on the lipidic profile and CRP as an inflammatory marker to suggest potential physiological pathways that mediate the observed genetic effects. We hypothesized that *APOE* genotypes would be implicated in differences in lipidic and inflammation profile, and these differences will be mediating the effect of *APOE* genotype on cognition.

1. MATERIALS AND METHODS

1.1 Sample /participants

The Barcelona-AsIA Neuropsychology Study (AsIA-NP) [39] is a prospective longitudinal study with 648 participants aged over 50 with a moderate–high (mean=8.11; SD =4.08) vascular risk in REGICOR [40].

Exclusion criteria were: Mini mental State Examination score <25, history of stroke or transient ischemic attack (TIA), coronary heart disease, chronic neurological disease, or severe psychiatric disorder; severe disability or institutionalization; and other medical diseases that

could affect cognitive assessment and function. Details of the recruitment process and sample protocol have been previously published [41]. Participants underwent a complete physical and cognitive evaluation including a blood extraction. This study has been approved by the University of Barcelona and the Germans Trias i Pujol University Hospital Ethics committee and is in accordance to the provisions laid down in the 1964 Declaration of Helsinki and its later amendments. All participants gave their informed written consent prior to their recruitment in the study.

1.2 Neuropsychological and behavioral assessment

A comprehensive neuropsychological battery was administered to all participants and subjects' raw test punctuations were standardized to Z scores. A factorial analysis, already published [39], resulted in three factors that were labelled as: (1) Visuospatial skills/speed, (2) Verbal Memory, and (3) Verbal Fluency (Suppl. Table 1). Current depressive symptoms were assessed with the *Short (15-items) Geriatric Depression Scale* (GDS-15) [42] with scores higher than 5 being indicative of probable depression.

1.3 Genetic and biological analyses

Blood samples were taken following an overnight fast. They were drawn, processed, and stored in a biobank at -80°C. Concentrations of high-density-lipoprotein cholesterol (HDL-C), triglycerides (TG) and TC were determined using standardized automated high throughput enzymatic methods and LDL-C levels were calculated using Friedewald formula. CRP measurement was carried out with a nephelometric method (Delta, Radim Iberica). Details of biological analysis have been previously described elsewhere [39].

Genomic DNA was extracted from whole blood using an ISOLATE II Blood DNA Kit (BIOLINE, UK). Three single-nucleotide polymorphisms (SNPs) in the *APOE* (rs429358 and rs7412) and *BDNF* (rs6265) genes were genotyped using KASPar assays, an allele-specific PCR technology, by an external genotyping core facility (Progenika Biopharma S.A., Spain). Negative controls (blanks) and sample duplicates were included for quality control. Further details on the SNPs analyzed are detailed on Suppl. Table 2. *APOE* alleles for the two SNPs were recoded as *ε2, ε3*, and *ε4* following standard nomenclature (Suppl. Table 3).

The effect of age on cognition on any of the genetic variants was analyzed grouping years by decades [43] and a complementary analysis was also done grouping by 5 years.

1.4 Statistical analyses

For demographic and clinical data, we used the Statistical Package for Social Sciences version 24 (SPSS Inc., Chicago, IL, USA). Normality distribution of the data was tested with a *Shapiro–Wilk test* before each analysis. Sex differences in demographic and behavioral characteristics were analyzed as follows: independent two-sample t-tests for normally distributed continuous variables; *Mann–Whitney U-test* for non-normally distributed continuous variables; and chi-square tests for categorical variables. Quality control on genetic data was performed by removing samples with more than one genotype missing and by assessing *Hardy-Weinberg equilibrium* expectations in our sample. All sample duplicates and blanks were adequately genotyped.

For the cognitive domains, linear regression analyses were performed between the three domains (Visuospatial skills/speed, Verbal Memory and Verbal Fluency) included as dependent variables and the *APOE* and *BDNF* genotypes as independent variables. For the *APOE*

polymorphism, allele effects were analyzed comparing ε2 vs non-ε2 alleles and ε4 vs non-ε4 alleles under additive genetic models. Additional analyses studied the effect of the ε2 and ε4 alleles compared only to the ε3 allele, which was taken as the reference allele, to better disentangle the effects of the different alleles. Those analyses were executed using the specialized software for genetic association analyses PLINK 1.9 (www.cog-genomics.org/plink/1.9/). For these analyses, age, sex, years of education, depression and REGICOR score were included as covariates in all regression models. Covariates were selected based on previous analyses on this cohort [39]. Regressions were conducted under an additive genetic model. *Post-hoc* regressions were also performed on tests belonging to each significant cognitive domain. Interactive effects between *APOE* genotypes, sex and age on cognitive domains were also assessed. Genetic interactions between *BDNF* and *APOE* alleles were analyzed. Permutation testing (1000 permutations) was implemented on significant results to correct for Type I error and to assess their robustness. Additionally, we implemented the Bonferroni method to control the familywise error rate (FWER). Considering all association tests performed (N=72), a FWER-corrected alpha of 0.000694 was determined. This method provides error control that is exact only for independent tests, but it is overly conservative for tests that are stochastically dependent, as in the case of our study.

Post-hoc analyses were computed as well to identify biomarkers that might mediate the observed genetic effects on cognition. First, we compared the lipidic profile (TC, HDL-C, and LDL-C) and the CRP levels between carriers of different alleles identified in the *ad hoc* analyses for the whole sample and stratified by the significant interaction variables. Variables that showed significant differences were used as input for the mediation analyses to better understand the biological differences involved in the heterogeneity of cognitive trajectories linked to *APOE*

genotypes. 5000 bootstrap samples were run with a 95% confidence interval using the INDIRECT macro for SPSS. Indirect effects were interpreted as significant if the confidence interval did not include zero [44]. Genotype was the independent variable in all mediation analyses, vascular risk factors (TC, LDL-C, and CRP) were used as mediators, and cognitive domains that showed significant associations in the *ad-hoc* analyses were the dependent variables. All models included age, years of education as covariates and, in the whole sample models, sex was also included.

2. RESULTS

2.1. Subjects

Out of the 747 individuals in the original AsIA-NP study, blood samples were available for 684 participants. Thirty-six subjects were further excluded due to genetic information of bad quality (>1 SNPs with missing information). The final sample comprised a total of 648 subjects, with a mean age of 66.1 years (SD = 7.6) and 6.3 (SD = 4.2) years of education on average. The 65.9 % of participants were males. Summary details of the sample are described in Table 1.

After removing participants with a low call rate, the total genotyping rate in the 648 remaining individuals was 0.98. Genotype frequencies for all markers did not differ significantly from Hardy–Weinberg equilibrium expectations. Allele frequencies for the *APOE* gene were 4.7%, 85.3%, and 9.8% for the *ε2, ε3* and *ε*4 alleles, respectively. For the *BDNF* polymorphism, the minor allele was Met, with a frequency of 21.6%. Genotype frequencies are reported on Suppl. Tables 2 and 3.

[Insert table 1 about here]

2.2 Genotype and cognitive domains

Linear regression models for association between *APOE* and *BDNF* polymorphisms with cognitive domains showed that *APOE ε*2-carriers, a total of 57 subjects (27 females and 30 males) had better performance in the verbal Memory (p = 0.002) and Fluency (0.001) Domains (Table 2 and Figure 1A and 1C). The significance of these associations was confirmed through a permutation test after 1000 permutations. Other associations between *APOE ε*4-carriers and *BDNF* Met carriers with the Visuospatial Skills and Speed and the Fluency Domains, respectively, were found but they did not hold after a permutation test (Table 2).

[Insert table 2 about here]

Associations for the *APOE ε2* allele were further confirmed individually for every test belonging to the Memory and Fluency Domains (Word List-immediate recall (p = 4.264 $^{e-04}$); Word List-delayed recall (p = 0.004), and Phonemic (p = 0.010) and Semantic Fluency (p = 0.025)) (Suppl Table 4). Associations of the individual tests in the Memory Domain, but not in the Fluency Domain, remained significant after 1000 permutations.

When these analyses were repeated comparing *APOE ε2* and *ε4* alleles only to the ε3 reference allele, results did not substantially differ.

2.3 Gene-environment interactions on cognitive function

2.3.1 Sex and age

The analysis of interactions between genotype and sex on cognitive domains revealed significant interactions of sex with the *APOE* $\varepsilon 2$ allele (p = 0.004) in the Verbal Memory Domain and a suggestive trend in the Fluency Domain (p = 0.094) (Suppl Table 5). Post-hoc analyses were performed separately by sex revealing a remarkable protective effect of *APOE* $\varepsilon 2$ in the performance of Verbal Memory and Fluency tasks among females, but not in males (Suppl Table 6, Figure 1B and 1D, and Suppl Table 7). For every $\varepsilon 2$ allele that they carried, women showed increases of 0.74-0.83SD and 0.53-0.57SD in the standardized scores in the Verbal Memory ($p = 3.167^{e-05}$) and Fluency (1.853^{e-04}) Domains. These results were confirmed after the permutation test ($p = 9.999^{e-04}$ and p = 0.004, respectively) and reached also the Bonferroni-corrected significance threshold.

[Insert Figure 1 about here]

Association analyses conducted for the individual tests in these cognitive domains confirmed a higher performance among women carrying $\varepsilon 2$ alleles. All individual tests were nominally significant and were confirmed after 1000 permutations, except for the phonemic fluency test, that only showed nominal significance (Suppl. Table 8).

We did not observe any interactive effect of age and any of the genetic variants analyzed on cognition grouping years by decades (Suppl Table 9). A complementary analysis was done grouping age by 5 years with the same negative results (data not shown).

2.3.2. Cardiovascular risk factors

When we compared *APOE* carriers and non-carriers in the whole sample, *APOE* $\varepsilon 2$ carriers showed significantly lower levels of TC $t_{(629)} = 2.293$; p = 0.022, LDL-C levels $t_{(629)}$

= 2.464; p = 0.016 and CRP $t_{(633)}$ = 2.607; p = 0.010). No significant differences were found for HDL-C. We also found a significative interaction between sex and TC ($F_{(1)}$ = 8.502; p = 0.004) and a tendency with HDL ($F_{(1)}$ = 3.747; p = 0.053). When we analyzed separately males and females, lower levels of TC $t_{(18.74)}$ = 2.114; p = 0.040, LDL-C $t_{(36,83)}$ = 1.975; p = 0.056 and CRP $t_{(50,61)}$ = 2.421; p = 0.019 in ε2 carriers (n = 27) comparing with non-carriers (n =193) were found only among females.

In order to provide candidate physiological pathways that might be mediating the genetic effect detected for the *ε2* allele on Verbal Memory and Fluency Domains, we conducted mediation analyses using TC, LDL-C and CRP in the whole group, and in males and females separately. For the whole sample, TC and LDL-C were found to partially mediate the effect of the *APOE ε2* allele on Verbal Memory Domain (Figure 2). When the same mediation analysis was performed for males and females separately, TC partially mediated the effect of the *APOE ε2* allele on Verbal Memory, but only in the female group (Figure 3).

[Insert Figures 2 and 3 about here]

2.3.3 Gene-gene interactions on cognitive domains.

Interaction analyses revealed some gene-gene interactive effects between *BDNF* and *APOE ε2* in the Visuospatial and Speed Domain (p =4.770 $^{e-04}$) (Suppl Table 10). Specifically, subjects who carried *BDNF Met* alleles performed worst on the Visuospatial and Speed Domain only if they were also *APOE ε2* carriers and carriers of other *APOE* alleles did not show differences in their performance regardless of their *BDNF* genotype (Suppl Table 11).

3. DISCUSSION

We investigated the effect of the *APOE* and *BDNF* genotypes on cognition in a non-demented over 50 population-based sample and their genetic and environmental interactions. We also analyzed the effects of *APOE* on cardiovascular risk factors. Specifically, we studied lipidic profile and inflammation, two variables that play a relevant role in cognitive aging and that are related with the *APOE* gene [26]. We suggested potential physiological pathways that may mediate the observed genetic effects. Our main result was that there are sex differences in the role of *APOE* on cognition. Specifically, we found that *APOE ε2* shows a neuroprotective role in the Verbal Memory and Fluency Domains in healthy over 50 females but not in males. As far as we know, this is the first study to identify a sex-specific protective effect of *APOE ε*2 on cognitive performance in an adult community aged 51 to 91. We also found relevant sex-specific differences in CRP and lipidic and profile between *APOE* genotypes. Furthermore, TC and LDL-C were partially mediating the relationship between *APOE* genotype and cognition. Finally, we reported a gene-gene interaction between the *BDNF* and *APOE* genes.

Our results revealed that *APOE ε*2 carriers perform significantly better in the Verbal Memory and Fluency Domains. This data is relevant because these domains are the primarily affected in aging [45]. There is not a consensus in the few published studies about the effects of *ε*2 on cognitive function [46,47]. Our finding supports previous literature suggesting a protective effect of *ε*2 on cognition [48,49]. Negative results have been, however, also reported on literature [50,51]. These negative results could be explained by the large age-range in their studies (between 44 and over 90 and 18 and 90 respectively) and the antagonistic pleiotropic effect of *APOE* across lifespan [13]. Contrary to our expectations, but consistent with Sinclair et al. (2017), we did not

find a detrimental effect of the *APOE* -ε4 nor *BDNF* Met alleles on cognition in our sample, maybe due to our sample characteristics [10]. The AsIA-NP cohort includes middle-aged cognitively healthy subjects with moderate-high vascular risk factors. In this scenario, a certain degree of survivor bias might have enriched our cohort with genetically protected middle-aged participants and might have excluded subjects with cognitively deleterious genetic background.

Environmental and genetic variables are implicated in the effect of *APOE* on cognition. In our sample, sex, lipidic profile, CRP, and a gene-gene interaction with the *BDNF* gene were found as relevant variables. Nevertheless, our results did not support a differential age-effect of the *APOE* genotype on cognition maybe because of the limited age range and the sample characteristics: cross-sectional assessment in a group of non-cognitively impaired participants.

Interestingly, interactions between sex and *APOE* evidenced that the neuroprotective role of *APOE* ε2 on the Verbal Memory and the Fluency Domains was found only in the female subgroup with a substantial genetic effect in the Verbal Memory and Fluency Domains for every ε2 allele carried (~0.75-0.8SD and ~0.5SD, respectively). These effects are remarkable since a ε2/ε2 homozygote female would show a performance in the Verbal Memory domain 1.5SD higher on average than an ε3/ε3 subject, a difference that is usually considered clinically relevant [52,53] (and 1SD in the Fluency Domain). To our best knowledge, no studies to date have focused on the interactive effects of sex**APOE* ε2 and cognitive performance in a community of this age group. The literature focused on the interactive effects of sex**APOE* ε4 on cognitive performance in this population is very limited [22,54]. In the study conducted by [54], the authors found that *APOE* ε4 allele was associated with cognitive decline only in women, pointing out to the effect of sex on *APOE* ε4 allele while in the study by [22] the authors found that non-ε4 male carriers showed worse memory and less adjusted hippocampal volume. Studies regarding the

increased probability of *APOE* ε4 carriers of developing dementia found sex as a key factor to understand the effect of this gene on cognitive decline with female ε4-carriers at higher odds of developing AD than their male counterparts [4,19]. Those studies and others have highlighted a greater detrimental impact of the *ε4* allele in women for different AD biomarkers, such as cerebrospinal fluid (CSF) and brain tau deposition [20,55], amygdala and hippocampi volumes [19] and increased general AD risk [21]. Possible mechanisms that may explain *APOE* sexual dimorphism over cognition are vascular risk factors, menopausal and perimenopausal hormonal changes, inflammation and the complex interaction between them [56]. When we studied differences in lipidic profile and inflammation in the whole sample we found that *APOE* genotype was associated with differences in lipidic profile and inflammation levels, with lower TC, LDL-C and CRP levels in APOE ε2 carriers compared with ε2 non-carriers. When we analyzed those effects according to sex, differences between *APOE* genotypes in lipidic profile and inflammation were found only in females as previously observed for cognition. Furthermore, TC partially mediated the neuroprotective effect of *APOE* ε2 on verbal memory in the whole sample and in females but not in males, suggesting a potential biological pathway to explain sex differences in the neuroprotective role of *APOE* on this cognitive domain. Differences in the effects of *APOE* genotypes on lipids are currently proposed to be a mechanistic link between *APOE* polymorphism and cognition, possibly through Aβ aggregation, deposition, and clearance [57]. In fact, it has been stated that *APOE* ε4 carriers have lower HDL-C levels and higher LDL-C levels in plasma in comparison with non-ε4 carriers [58]. However, the relationship between *APOE* genotypes, lipid profile, and cognition has not been always confirmed across different studies (Bojar et al., 2015), probably due to the different effect that TC has across lifespan [59] and because TC score ignores the different contribution of HDL-C and LDL-C.

CRP, a marker of inflammatory processes, is another variable that might be involved in the development of cognitive impairment. Our results support the idea that *APOE* might modify the relationship between CRP and cognitive function. In the same line agreement, Bojar et al. (2016) reported that women *ε4/ε4* homozygotes had higher CRP values associated to lower cognitive results [60].

Finally, we found a gene-gene interaction between variants in the *APOE* and *BDNF* genes. Specifically, *BDNF* showed significant interactions with the *APOE ε2* allele on the Visuospatial/speed Domain and *BDNF Met* carriers performed worst in this domain if they were also carrying an *APOE ε2* allele*ε*. These interactions should be explored in future research since our sample size limited the conclusions that could be extracted from stratified analyses.

Taken together, our findings are relevant in different aspects. First, our results highlight the sex-specific neuroprotective role of *APOE ε2* on cognition in a middle-aged sample with moderate–high vascular risk factors. Second, in our cohort, the neuroprotective role of *ε2* is remarkable among women and absent, or too subtle to be detected, among men. Finally, this differential effect might be mediated through the modulation of cardiovascular risk factors (evidenced in our study through changes in TC and LDL-C levels) and the inflammation profile (assessed through CRP levels).

Moreover, other genes (i.e. *BDNF*) also contribute to the observed interindividual differences interacting together. However, some limitations of our study need to be considered. Most associations in our study withheld permutation tests and several of them (e.g. the *ε2* allele sex-specific effects on verbal memory and fluency and the *BDNF x APOE* interaction) remained significant after a Bonferroni correction even if all ad hoc analyses were considered (an overly conservative approach since several comparisons are not independent). However, the low

frequency of the *APOE ε2* allele still limits the statistical power of the analyses and may have biased the results. Moreover, the cardiovascular profile of our sample and the non-demented status could have biased some of our results and finally, although the age span of our participants almost fell entirely in the post-menopausal range, and we did not find age differences between *ε2* female carriers and non-carriers, the absence of hormonal information did not allow to confirm if the sex-specific effects of *APOE ε2* are due to hormonal changes during menopause. Future studies with bigger *APOE ε2* samples and different populations are needed for a deep understanding of *APOE* implications in cognition.

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Tables

Table 1. Demographic, clinical and neuropsychological characteristics of the sample

	WHOLE SAMPLE (n = 648; 100%)				FEMALES (n = 223; 34.41%)				MALES (n = 492; 65.59%)			
	M	**Min.**	**Max.**	**SD**	**M**	**Min.**	**Max.**	**SD**	**M**	**Min.**	**Max.**	**SD**
Demographic data												
Age (years)	66.1	51.0	91.0	7.6	63.6	51.0	89.0	6.9	67.4	52.0	91.0	7.7
Education (years)	6.3	0.0	24.0	4.2	6.1	0.0	17.0	3.5	6.5	0.0	24	4.5
Clinical data												
GDS-15	2.3	0.0	15.0	2.4	3.2	0.0	15.0	3.1	1.9	0.0	14.0	1.9
REGICOR score (0-23)	8.1	1.0	29.0	4.1	6.2	1.0	17.0	2.39	9.1	2.0	29	0.7
Total cholesterol (mg/dL)	207.5	69.0	350.0	40.2	213.5	104.0	350.0	43.1	204.4	69.0	348.0	38.3
HDL-C (mg/dL)	54.6	110.0	15.0	11.7	56.9	36.0	110.0	11.94	53.4	15.0	105	11.4
LDL-C (mg/dL)	152.9	54.0	297.0	37.0	156.6	63.0	278.0	39.91	151.0	54.0	297	35.3
CRP (mg/L)	4.3	0.0	91.90	7.4	0.6	0.0	2.0	0.66	4.6	0.0	91.9	8.7
Neuropsychological data												
Visuospatial skills/speed domain												
Visual reproduction-immediate recall (WMS-III)	0.00	-2.84	2.09	0.99	-0.06	-2.84	2.09	0.91	0.03	-2.78	2.09	1.03
Visual reproduction-delayed recall (WMS-III)	-0.00	-1.54	2.78	0.99	-0.01	-1.54	2.36	0.96	0.01	-1.54	2.78	1.01
Visual reproduction-copy (WMS-III)	-0.00	-8.17	1.31	0.98	0.02	-8.17	1.21	1.10	-0.01	-4.70	1.31	0.91

Digit Symbol Coding (WAIS-III)	-0.00	-1.93	3.16	0.98	-0.08	2.66	-1.62	0.84	0.04	-1.93	3.16	1.04
Grooved Pegboard Test (preferred hand)	-0.15	-1.16	3.77	0.78	0.60	-1.30	7.88	1.04	-0.12	-1.30	7.88	0.96
Trail Making Test Part A	-0.00	-1,19	7.96	1.00	0.11	-1.17	7.96	1.11	0.06	-1.19	5.44	0.92
Verbal Memory domain												
Word list-immediate recall (WMS-III)	0.00	-2.86	3.24	1.00	0.15	-2.01	2.73	0.96	-0.08	-2.86	3.24	1.01
Word list-delayed recall (WMS-III)	-0.00	-2.02	2.76	1.00	0.15	-2.02	2.36	1.02	-0.08	-2.02	2.76	0.97
Verbal Fluency domain												
Letter fluency (P)	-0.00	-2.46	3.80	1.00	-0.11	-2.46	2.24	0.88	0.05	-2.46	3.80	1.03
Semantic fluency (animals)	-0.00	-2.80	3.78	1.00	-0.13	-2.59	2.76	0.93	0.07	-2.80	3.78	1.02

M = Mean; Min. = Minimum; Max. = Maximum; SD = standard deviation; HDL-C = High-density lipoprotein; LDL = Low-density lipoprotein; CRP = C-reactive protein. Neuropsychological data is expressed in Z scores.

Table 2. Model 1. Linear regression results for association between *APOE* and *BDNF* polymorphisms and Z-scores for cognitive domains in the AsIA_NP cohort.

			VISUOSPATIAL/SPEED				VERBAL MEMORY				VERBAL FLUENCY			
SNP	**Model**	**n**	**β**	**95%CI**	**p**	**p-perm**	**β**	**95%CI**	**p**	**p-perm**	**β**	**95%CI**	**p**	**p-perm**
APOE	**ε4 vs. non-ε4**	632	0.149	0.015 / 0.284	0.030	0.178	-0.126	-0.286 / 0.334	0.122	0.540	0.044	-0.106 / 0.193	0.568	1
APOE	**ε4 vs. ε3**	570	0.136	-0.003 / 0.275	0.057	0.189	-0.103	-0.267 / 0.060	0.216	0.574	0.021	-0.131 / 0.174	0.078	0.944
APOE	**ε2 vs. non-ε2**	632	-0.124	-0.313 / 0.064	0.197	0.732	0.348	0.126 / 0.570	0.002	0.017	0.342	0.135 / 0.550	0.001	0.010
APOE	**ε2 vs. ε3**	510	-0.122	-0.322 / 0.078	0.233	0.604	0.343	0.343 / 0.112	0.004	0.016	0.284	0.069 / 0.499	0.010	0.038
BDNF	**Met vs. Val**	641	0.084	-0.017 / 0.185	0.105	0.490	-0.073	-0.193 / 0.047	0.233	0.811	0.135	0.023 / 0.247	0.018	0.141

Analyses have been run under an additive (i.e. allele-dose dependent) genetic model. Age, sex, years of education, depression, and REGICOR score have been included as covariates in all analyses. Beta coefficients (and 95% CI) represent the effect of each extra minor allele. P-perm: probability of the observed p-values after 1000 permutations.

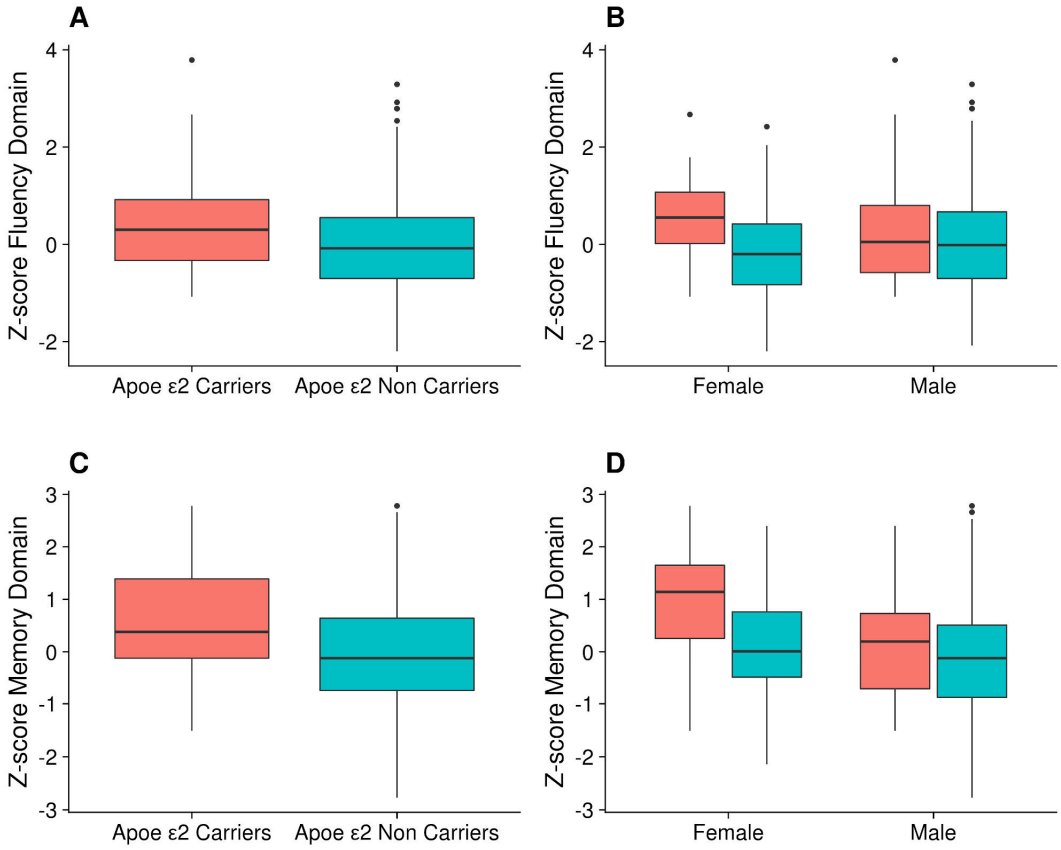
Figure captions

Figure 1. Differences in Fluency Domain and Verbal Memory Domain between $\varepsilon 2$ versus non $\varepsilon 2$ carriers: A ($p = 0.001$), C ($p = 0.002$). Differences in Fluency and Verbal Domain between $\varepsilon 2$ versus non $\varepsilon 2$ carriers according to sex: B (females: $p= 1.853$ e-04; males: $p = 0.165$), D (females: $p= 3.167$ e-05; males: $p =0.633$).

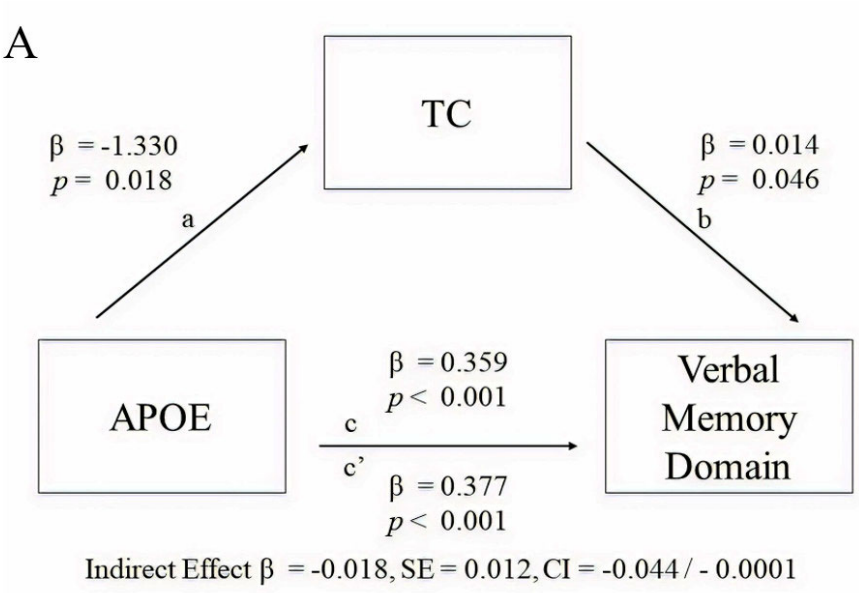
Figure 2. Mediation models between *APOE* $\varepsilon 2$ status ($\varepsilon 2$ carriers vs. non-carriers), TC (A) or LDL-C (B) and Verbal Memory in the sample (n=629). Path A is a regression between the independent variable *(APOE)* and the mediator (TC and LDL-C respectively). Path B analyzes the effect of the mediator over the dependent variable (Verbal Memory Domain), path C tests if *APOE* predicts the score in Verbal Memory Domain, path C' reflects the association between *APOE* and the score in Verbal Memory Domain and finally, the indirect effect reports the effect of the mediator in the model. Results indicate that TC and LDL-C partially mediate the relation between *APOE* genotype and verbal memory performance. Age, sex, and years of education were included as covariates.

Figure 3. Mediation model between *APOE* $\varepsilon 2$ status ($\varepsilon 2$ carriers vs. non-carriers), TC and Verbal Memory in the female sample (n=219). Path A is a regression between the independent variable *(APOE)* and the mediator (TC). Path B analyzes the effect of the mediator over the dependent variable (Verbal Memory Domain), path C tests if *APOE* predicts the score in Verbal Memory Domain, path C' reflects the association between *APOE* and the score in Verbal Memory Domain and finally, the indirect effect reports the effect of the mediator in the model.

Results indicate that TC partially mediates the relation between the APOE genotype and verbal memory performance. Age, sex, and years of education were included as covariates.

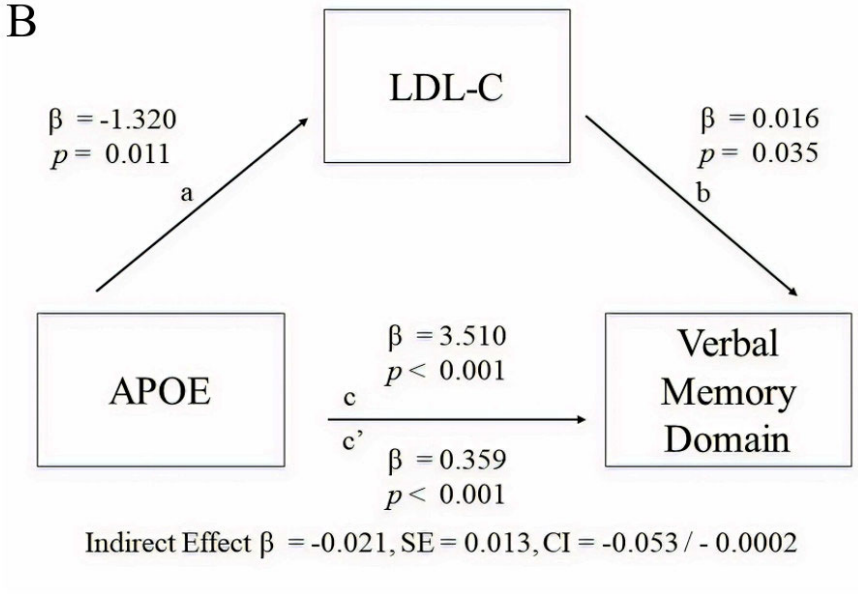


A

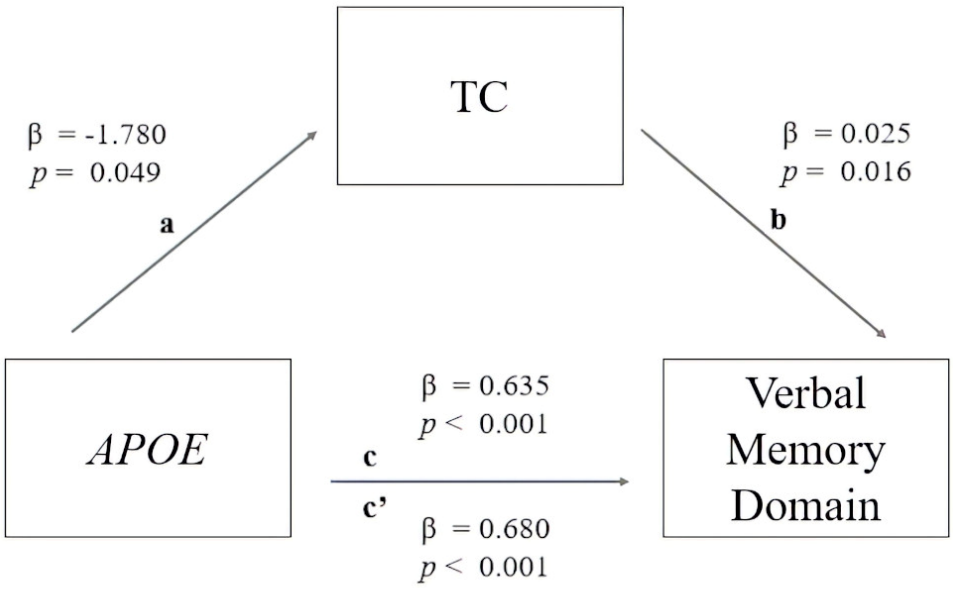


Indirect Effect β = -0.018, SE = 0.012, CI = -0.044 / - 0.0001

B



Indirect Effect β = -0.021, SE = 0.013, CI = -0.053 / - 0.0002



Indirect Effect β = -0.452, SE = 0.026, CI = -1.023 / -0.002