Dementia and Geriatric Cognitive Disorders

Dement Geriatr Cogn Disord 2017;44:328–342
DOI: 10.1159/000481852
Accepted: September 28, 2017
Published online: February 7, 2018

Original Research Article

APOE ε4 Genotype and Cognitive Reserve Effects on the Cognitive Functioning of Healthy Elders

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Keywords
APOE genotype · Healthy aging · Cognitive performance · Age · Educational level

Abstract
Aim: To test the association between cognitive performance and *APOE* genotype, and to assess potential modifications of this association by sociodemographic and neuroanatomical factors in a sample of 74 healthy elders. ***Methods:*** Firstly, we explored the isolated role of the *APOE* ε4 genotype (i.e., APOE4) in different neuropsychological tests, and then the effects of its interaction with sociodemographic (i.e., age, gender, and educational level) and neuroanatomical (i.e., hippocampal volumes) variables. Subsequently, we performed the same analyses after dividing the sample into two subgroups according to their Mini-Mental State Examination scores (control-high group ≥29 and control-low group <29). ***Results:*** In the whole group, APOE4 carriers exhibited a significantly poorer execution in several cognitive domains including global cognitive functioning, episodic memory, verbal fluency, and naming. This effect was more noticeable in older and less educated subjects. The separated analyses revealed that APOE4 carriers in the control-low group exhibited lower scores in global cognitive functioning and episodic memory, while no effects were observed in the control-high group. Nei-

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Dement Geriatr Cogn Disord 2017;44:328–342
DOI: 10.1159/000481852

ther gender nor hippocampal volumes showed a significant interaction effect with APOE genotype. ***Conclusions:*** Current results point out that APOE4 genotype influences healthy aged cognition, although factors such age or educational attainment seem to modulate its effects.

Introduction

There is a growing interest in exploring the factors that may contribute to "successful" or "unsuccessful" cognitive aging. For example, Apolipoprotein E (*APOE*) genotype, particularly the ε4 allele (i.e., APOE4), has been deemed a risk factor for the development of late-onset Alzheimer disease (AD) and also, in broad terms, an agent that contributes to "unsuccessful" aging [1]. Several studies demonstrated that APOE4 carriers showed an impaired performance on cognitive testing as compared with noncarriers, especially in the memory domain (for a review, see [2]). Such a deleterious effect is well established in AD and mild cognitive impairment (MCI) patients, but it was more elusive in the healthy aged [3]. In this regard, two main hypotheses concerning the influence of the APOE genotype on healthy aged cognition have been proposed: (a) the so-called preclinical/prodromal hypothesis [4] and (b) the cognitive phenotype hypothesis [5, 6]. The preclinical/prodromal hypothesis affirms that any differences in cognitive performance that emerge in healthy APOE4 carriers are due to an increased number of nondetected preclinical or prodromal AD cases in this group. Hence, if those cases were eliminated, differences would disappear. In contrast, the cognitive phenotype hypothesis posits that the ε4 allele induces neural damage that accumulates over time leading to cognitive impairment, and therefore APOE4 carriers would exhibit some cognitive deficits irrespectively of the development of AD. Some evidence exists supporting both hypotheses, and consequently the influence of APOE genotype on the mental status of healthy aged is probably one of the most relevant controversies in this field of investigation (see for example [7–10]).

The APOE genotype is one of the multiple factors that may affect cognitive aging. Nevertheless, as the interactions between age [11], gender [12], hippocampal atrophy [12] and the APOE genotype may induce changes in cognitive performance, the complexity of this debate increases. Additionally, educational attainment plays an important role in successful aging and has been related to the concept of cognitive reserve (CR) [13]. It is well documented that a higher educational attainment in earlier life reduces the incidence of dementia (see for example [12, 14, 15]); however, some evidence questioned the role of CR, and particularly of educational attainment, as an active agent. For instance, Stern et al. [16] noted that healthy subjects with lower educational attainment may perform worse on neuropsychological testing, and therefore their scores would be closer to the dementia cutoff points. Notably, education *per se* had no conclusive effects on the rate of cognitive decline of highly educated controls [17]. These interesting results suggested that education might be "confounding" mental status evaluation, according to Katzman's [18] terminology. As a result, studies in healthy population are critical to understand how education might influence the process of cognitive deterioration.

With this background in mind, we performed an investigation to test the association between cognitive performance and APOE genotype, and also to assess potential modifications of this association by sociodemographic and neuroanatomical factors. To reach this goal, a two-step procedure was adopted. First, the individual and potentially interactive effects of APOE genotype, age, educational attainment, hippocampal volume, and gender on

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Table 1. Demographics and relevant characteristics of the whole sample (n = 74)

Age, years	70.36±4.76
MMSE	28.82±1.45
Males/females	27/47
Education, years	13.56±4.96
APOE4 noncarrier/carrier	45/29 (1 homozygote)
RH-ICV	0.00240±0.00036
LH-ICV	0.00239±0.00037

Data are presented as mean ± SD or *n*. MMSE, Mini-Mental State Examination; APOE, gene encoding for apolipoprotein E; APOE4 noncarrier, there is not any ε4 allele; APOE 4 carrier, there is at least 1 ε4 allele; LH-ICV, left hippocampal volume/intracranial volume; RH-ICV, right hippocampal volume/intracranial volume.

cognitive performance were assessed in a sample of healthy aged individuals. In a second step, and considering the possibility that the influence of some undetected MCI or preclinical AD cases may actually be biasing cognitive performance, the whole sample was split into two groups according to the Mini-Mental State Examination (MMSE) [19] scores following recently proposed criteria (see below). Once the groups were separated, APOE4 effects were re-evaluated. The intention of such strategy was to increase the certainty that cognitive performance had a low probability of being affected by "hidden" cognitive deterioration cases in at least one group.

Methods

Participants

Seventy-four community-dwelling volunteers (47 females) were recruited from the "Seniors Center of Chamartín District," Madrid, Spain. All of them were right-handed [20] and native Spanish speakers. Demographic and relevant variables are shown in Table 1.

All subjects underwent a clinical evaluation, magnetic resonance imaging (MRI), and genetic analysis. In order to evaluate the global cognitive and functional status of the participants, all of them were screened by a set of standardized tests (for a review of the protocol, see [21]). Additionally, participants underwent an extensive neuropsychological assessment to explore their cognitive functioning by using the following tests: direct and inverse digit span test (DDS and IDS, Wechsler Memory Scale III, WMS-III) [22], immediate and delayed recall (IR and DR, WMS-III) [22], phonemic and semantic fluency (PhF and SF, controlled oral word association test) [23], ideomotor praxis of Barcelona test [24], visual object and space perception test (VOSP) [25], Boston naming test (BNT) [26], and trail-making test (TMT), parts A and B (TMT-A and TMT-B) [27].

All subjects were in good health, with no significant medical, psychiatric or neurological diseases (including complaints in the subjective cognitive domain, SCD). SCD was assessed using the Memory failures in everyday life following severe head injury [28] and the Subjective Cognitive Decline Questionnaire (SCD-Q) [29]. Although SCD has been recently included in the preclinical stage of AD [30, 31], its identification in the healthy aging population is essential [32] due to their association with a higher risk of AD [33, 34].

General inclusion criteria included age between 60 and 85 years, MMSE score ≥24, a modified Hachinski score ≤4 [35], a Geriatric Depression Scale Short-Form score ≤5, and a T2-weighted MRI within 12 months before the clinical evaluation without indication of infection, infarction, focal lesions or significant hippocampal atrophy. The study was approved by the Hospital Universitario San Carlos ethics committee, and all subjects gave informed consent prior to their participation in the study.

APOE Genotype

Genomic DNA was extracted from 10 mL blood samples in ethylenediaminetetraacetic acid. APOE haplotype was determined by analyzing single nucleotide polymorphisms (SNPs) rs7412 and rs429358 genotypes with TaqMan assays using an Applied Biosystems 7900 HT Fast Real Time PCR machine (Applied Biosystems, Foster City, CA, USA). A genotyping call rate over 90% per plate, sample controls for each

Table 2. Demographics and relevant characteristics of APOE4 noncarriers (*n* = 45) and APOE4 carriers (*n* = 29)

Variable	APOE4 noncarriers	APOE4 carriers
Age, years	69.60±4.25	71.55±5.32
MMSE	29.29±1.83	28.10±0.86
Males/females	15/30	12/17
Education, years	13.68±4.70	13.38±5.42
RH-ICV	0.00246±0.00283	0.00232±0.00044
LH-ICV	0.00246±0.00327	0.00230±0.00042

Data are presented as mean ± SD or *n*. MMSE, Mini-Mental State Examination; APOE, gene encoding for apolipoprotein E; APOE4 non-carrier, there is not any ε4 allele; APOE4 carrier, there is at least 1 ε4 allele; LH-ICV, left hippocampal volume/intracranial volume; RH-ICV, right hippocampal volume/intracranial volume.

genotype and negative sample controls were included in each assay. Three well-differentiated genotyping clusters for each SNP were required to validate results. Intra- and interplate duplicates of several DNA samples were included [36]. According to the presence or absence of the ε4 allele, participants were classified as APOE4 carriers or noncarriers (Table 1). The investigation did not include ε2 carriers due to its relatively selective role acting as a protective factor [37, 38] and since its prevalence in the population is low, we did not have a sufficient number of participants in our sample to form a group comparable to the groups of carriers and noncarriers of the ε4 allele. Demographic and relevant variables of APOE4 carriers and noncarriers are shown in Table 2.

Educational Attainment

Considering previous investigations that highlighted the role of educational attainment as a sensitive estimate of CR, this proxy was measured as years of formal education (range 1–25 years), and obtained by questioning participants or caregivers (see [21]).

MRI Acquisition and Hippocampal Volumes

3D T1 scans were collected with a General Electric 1.5-T magnetic resonance scanner, using a high-resolution antenna and a homogenization PURE filter (Fast Spoiled Gradient Echo sequence with parameters: TR/TE/TI = 11.2/4.2/450 ms; flip angle 12°; 1 mm slice thickness, 256 × 256 matrix; and FOV 25 cm). T1-weighted MRI images were then processed with Freesurfer software package (version 5.1.0) and its specialized tool for automated cortical and subcortical segmentation [39], in order to obtain the hippocampal volumes. This automated segmentation technique assigns a neuroanatomical label to each voxel in an MRI volume based on probabilistic information estimated from a manually training set of measurements. Hippocampal volumes were normalized considering total intracranial volume (ICV) to account for the variability in head volume along subjects. Accordingly, two variables were created and submitted for further analysis: LH-ICV = left hippocampal volume/ICV; and RH-ICV = right hippocampal volume/ICV.

Statistical Analyses

The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to assess the normality of all neuropsychological scores. When the normality assumption was violated, data were normalized using a log transformation. The transformed variables will be henceforth denominated by adding the "N" prefix to the name of the original variable, for example NTMTB-time and NBNT represent the transformed values of TMTB-time and BNT, respectively.

A two-step procedure was carried out. Firstly, a series of exploratory analyses were performed. Two-sample *t* tests were employed to evaluate differences in neuropsychological scores that may emerge due to APOE genotype influence in the whole sample (i.e., APOE4 carriers vs. noncarriers). Since this study focuses on the investigation of interaction effects, all continuous variables (i.e., age, years of formal education, and hippocampal volumes) were dichotomized according to their median values in order to provide a more straightforward interpretation of the statistical results. Hence, within-group homogeneity due to demo-

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graphical factors such as gender, age, and education was assessed by means of χ^2 tests. In the exploratory analyses, a level of $p \leq 0.1$ was fixed as a conservative screening criterion to select candidate variables for the generalized linear model (GLM). The use of this conservative level is based on outstanding works on this topic (see for example [40]). Secondly, a series of GLM univariate analyses were employed to investigate the combined effects of factors on each neuropsychological test score. GLM analyses were carried out by using univariate 2 × 2 ANOVA models, where the interaction between APOE genotype and: (1) dichotomized age; (2) dichotomized years of education; (3) gender; and (4) dichotomized left and right hippocampal volumes was assessed. On each of these models, the dependent variable was a neuropsychological test score, while APOE genotype, gender, dichotomized age, dichotomized years of formal education, and dichotomized hippocampal volumes were the fixed-effect factors. Post hoc pairwise tests were performed using Bonferroni correction.

As briefly noted in the introductory section, this two-step procedure was performed in the whole sample and in two subgroups of subjects. Such strategy was purposely adopted to test the preclinical/prodromal AD hypothesis of APOE4 effects on the cognitive performance of healthy aged. Previous studies accomplished this task using essentially two strategies: (1) the retrospective screening of those control cases who developed cognitive impairment in follow-up investigations [41–43]; and (2) the initial analysis of APOE4 effects in a combined sample of healthy controls and diagnosed MCI individuals, followed by a separated analysis within each group [44]. From our point of view, the second strategy might be more appropriate to test the hypothesis, but an even more restrictive approach can be adopted to avoid the "inadvertent intrusion" of prodromal AD cases. In this vein, MMSE scores ≥24 have been frequently utilized in the inclusion criteria of cognitive deterioration studies to select control subjects (see for example the ADNI2 initiative, http://adni.loni.usc.edu/) following the initial recommendations by Folstein et al. [19] that have been revised by Lopez et al. [45]. Importantly, a recent investigation by Roalf et al. [46] compared the accuracies of MMSE and the Montreal Cognitive Assessment (MoCA) as screening instruments for AD. Within this research background, the authors estimated those cut-off scores that optimized the discrimination between AD versus MCI cases, AD cases versus controls, and MCI cases versus controls. According to their findings, an MMSE score ≥29 yielded the highest discrimination capability to distinguish between cognitive deterioration and actual control cases. Following such basic information, we decided to split our whole sample into two groups, a subgroup formed by controls with MMSE scores ≥29 (henceforth called control-high group), and a second subgroup formed by controls with MMSE scores <29 (i.e., MMSE scores that ranged from 24 to 28 points, henceforth called control-low group). Using this very restrictive approach, we tried to minimize, as much as possible, the risk of including cases with subtle cognitive impairment in the control-high group. All statistical analyses were carried out using SPSS 22 statistical software.

Results

The Kolmogorov-Smirnov and Shapiro-Wilk tests showed that all scores satisfied the normality assumption (both $p > 0.2$) except for DDS, IDS, TMTA-time, TMTB-time, and BNT scores ($p < 0.01$). As a result, these five scores were log-transformed.

Relationship between Demographic Variables and APOE Genotype

χ^2 tests showed the homogeneity of dichotomized age by gender ($\chi^2_1 = 0.094$; $p = 0.759$). The same comparison by APOE genotype and dichotomized years of formal education showed no significant differences in both cases ($\chi^2_1 = 1.299$, $p = 0.254$, and $\chi^2_1 = 2.448$, $p = 0.120$, respectively). The comparison of gender distribution according to APOE genotype was not statistically significant ($\chi^2_1 = 0.493$; $p = 0.483$), indicating that the proportion of APOE4 carriers was homogeneously distributed in males and females. Finally, χ^2 tests indicated that the dichotomized years of formal education were homogeneously distributed according to both APOE genotype ($\chi^2_1 = 2.392$; $p = 0.122$) and gender ($\chi^2_1 = 0.406$; $p = 0.524$). However, although the χ^2 test was not significant, the proportion of less educated subjects was higher in the APOE4 carriers.

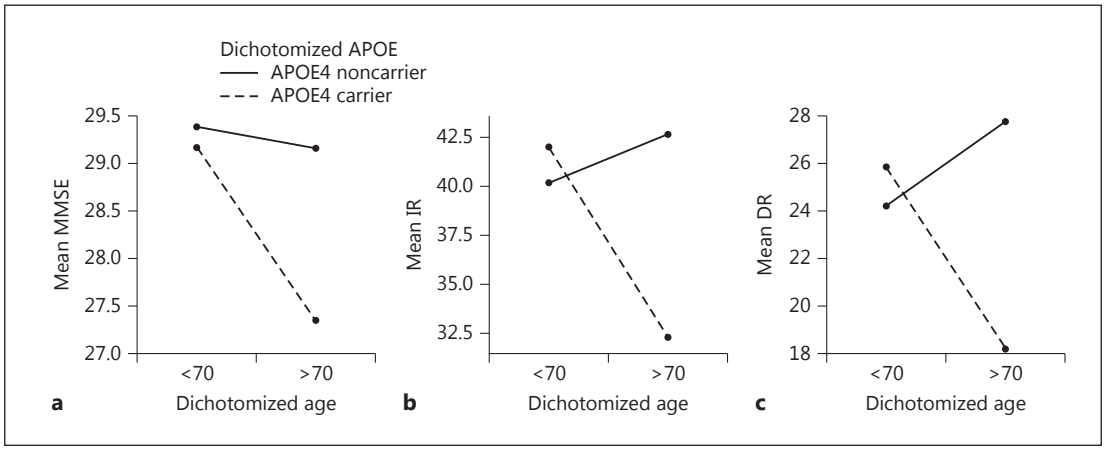

Fig. 1. Estimated means for APOE genotype and dichotomized age interaction effects in MMSE scores (**a**), IR scores (**b**), and DR scores (**c**).

APOE4 Effects in the Whole Sample

The exploratory analyses by means of two-sample t tests indicated that APOE genotype exerted an effect on several cognitive tests, including MMSE (t_{72} = 3.735; p = 0.0001), IR (t_{72} = 2.153; p = 0.035), DR (t_{72} = 2.403; p = 0.018), PhF (t_{72} = 2.513; p = 0.014), SF (t_{72} = 2.402; p = 0.019), NTMTB-time (t_{72} = –1.794; p = 0.07), and NBNT (t_{72} = 3.297; p = 0.002). In all tests, APOE4 carriers exhibited significantly lower scores than noncarriers, with the exception of NTMTB-time in which APOE4 carriers needed more time to complete the task.

As previously explained, these effects were further explored by means of univariate ANOVA models. The analysis of MMSE scores showed a significant effect of APOE genotype ($F_{1,70}$ = 11.782; p = 0.001), dichotomized age ($F_{1,70}$ = 11.988; p = 0.001), and the interaction between both variables ($F_{1,70}$ = 7.252; p = 0.001). Post hoc results indicated that older subjects carrying the APOE4 genotype showed significantly lower scores than noncarriers (p = 0.0001), while these effects were not significant in the younger group (p = 0.61). In carriers, the younger group showed significantly higher scores than the older ones (p = 0.03) (Fig. 1a). When genotype and education were analyzed together, results demonstrated significant effects of APOE genotype ($F_{1,70}$ = 16.019; p = 0.0001), dichotomized years of education ($F_{1,70}$ = 5.574; p = 0.02) and APOE genotype × dichotomized years of education ($F_{1,70}$ = 6.883; p = 0.011). Post hoc analyses indicated that subjects with less education carrying the APOE4 genotype showed significantly lower scores than noncarriers (p = 0.0001), while no differences were found in the higher education group (p = 0.26). In the carriers group, subjects with more years of education exhibited higher MMSE scores (p = 0.01), while this profile was not present in noncarriers (see Fig. 2a). Neither gender nor hippocampal volumes displayed an individual or interaction effect on MMSE scores.

IR was influenced by the principal effect of APOE genotype ($F_{1,70}$ = 3.786; p = 0.05), and by the APOE genotype × dichotomized age interaction ($F_{1,70}$ = 7.591; p = 0.007). Post hoc analyses revealed that older APOE4 carriers exhibited significantly lower scores than noncarriers (p = 0.001), while this effect did not appear in the younger group (p = 0.568). In the group of carriers, younger subjects had significantly higher scores than the older ones (p = 0.017) (Fig. 1b). The investigation of the combined effects of APOE and education indicated a significant individual effect of both variables ($F_{1,70}$ = 4.441, p = 0.039, for APOE genotype, and $F_{1,70}$ = 11.207, p = 0.001, for dichotomized years of education), and also a significant interaction effect ($F_{1,70}$ = 4.224; p = 0.046). Post hoc analyses indicated that subjects with less

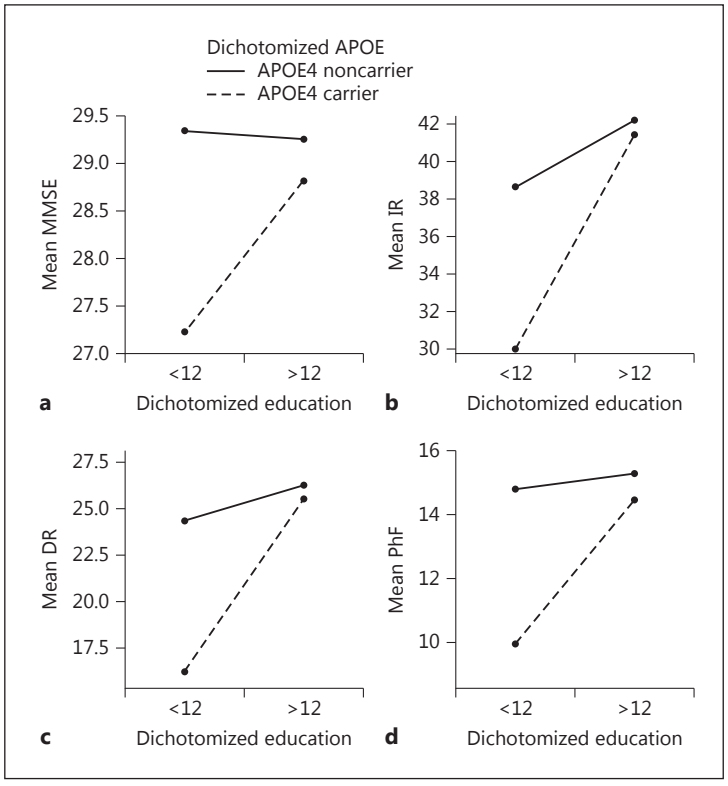

Fig. 2. Estimated means for APOE genotype and dichotomized years of education interaction effects in MMSE scores (**a**), IR scores (**b**), DR scores (**c**), and PhF scores (**d**).

education carrying the APOE4 genotype showed significantly lower scores than noncarriers ($p = 0.017$), while no differences were found in the higher education group ($p = 0.783$). APOE4 carriers with more years of education exhibited higher IR scores than less educated carriers ($p = 0.001$ (Fig. 2b). Paralleling IR results, DR scores were significantly affected by the individual effect of APOE genotype ($F_{1,70} = 5.153$; $p = 0.026$) and by the APOE genotype × dichotomized age interaction ($F_{1,70} = 10.275$; $p = 0.002$). Again, post hoc analyses revealed that older APOE4 carriers exhibited significantly lower scores than noncarriers ($p = 0.0001$), while this effect did not appear in the younger group ($p = 0.521$). Also, in the group of APOE4 carriers, the younger subjects exhibited significantly higher scores than the older ones ($p = 0.006$) (Fig. 1c) Additionally, the individual effects of APOE genotype ($F_{1,70} = 6.112$; $p = 0.016$), dichotomized years of education ($F_{1,70} = 9.759$; $p = 0.003$), and their interaction ($F_{1,74} = 4.216$; $p = 0.044$) exerted a significant influence on DR. Mirroring IR results, less educated subjects carrying the APOE4 genotype showed significantly lower scores than noncarriers ($p = 0.006$), while APOE4 carriers with more years of education exhibited higher DR scores than less educated carriers ($p = 0.001$) (Fig. 2c). Neither gender nor hippocampal volumes displayed an individual or interaction effect on IR and DR scores.

The analysis of PhF scores displayed slightly different results. Here, APOE genotype and age showed no significant interaction. However, the analysis of APOE and years of education revealed that both variables exerted an individual ($F_{1,70} = 6.671$, $p = 0.012$, for APOE genotype, and $F_{1,70} = 5.122$, $p = 0.027$, for dichotomized years of education) and also an interaction effect ($F_{1,70} = 3.322$; $p = 0.044$), showing identical tendency when compared with previous tests: less educated APOE4 carriers were characterized by a significantly worse performance in PhF as compared with less educated noncarriers (0.008). However, the effect was not significant in higher education groups ($p = 0.528$). Similarly, APOE4 carriers with more years of education exhibited higher PhF scores than less educated carriers ($p = 0.007$) (Fig. 2d). SF scores were

Table 3. Statistical results of the generalized linear models (GLM) for all the tests where APOE genotype exerted significant effects

Dependent variable	Factors interacting with APOE genotype	Principal and interaction effects	*F*	*p*	Post hoc analysis
Significant results of the GLM in the whole sample					
MMSE	Age	APOE genotype	11.782	0.001	
		Dichotomized age	11.988	0.001	
		APOE genotype × dichotomized age	7.252	0.001	0.0001
	Education	APOE genotype	16.019	0.0001	
		Dichotomized years of education	5.574	0.02	
		APOE genotype × dichotomized years of education	6.883	0.011	0.0001
IR	Age	APOE genotype	3.786	0.05	
		APOE genotype × dichotomized age	7.591	0.007	0.001
	Education	APOE genotype	4.441	0.039	
		Dichotomized years of education	11.207	0.001	
		APOE genotype × dichotomized years of education	4.224	0.046	0.017
DR	Age	APOE genotype	5.153	0.026	
		APOE genotype × dichotomized age	10.275	0.002	0.0001
	Education	APOE genotype	6.112	0.016	
		Dichotomized years of education	9.759	0.003	
		APOE genotype × dichotomized years of education	4.216	0.044	0.006
PhF	Education	APOE genotype	6.671	0.012	
		Dichotomized years of education	5.122	0.027	
		APOE genotype × dichotomized years of education	3.322	0.044	0.008
SF	None	APOE genotype	3.374	0.038	
NTMTB-time	Age	Dichotomized age	13.958	0.0001	
	Education	Dichotomized years of education	11.020	0.001	
NBNT	None	APOE genotype	9.125	0.002	
		Dichotomized age	4.867	0.031	
		Dichotomized years of education	12.296	0.001	
		Gender	7.543	0.008	

These analyses were performed for the whole sample.

exclusively influenced by APOE genotype effects ($F_{1,70} = 3.374$; $p = 0.038$). Neither gender nor hippocampal volumes displayed an individual or interaction effect on PhF and SF scores.

With regard to NTMTB-time, this test showed a quite different behavior that might be expected considering APOE genotype significance level on the t test. In this case, subjects' performance was not influenced by the interaction of APOE genotype with age or education ($p > 0.5$ in both cases). In contrast, variations in NTMTB-time scores were better explained by the direct effects of dichotomized age ($F_{1,70} = 13.958$; $p = 0.0001$) and years of education ($F_{1,70} = 11.020$; $p = 0.001$), while the age × years of education interaction was not significant. This indicates an additive rather than multiplicative relationship between both variables, demonstrating that performance in this test was poorer in older, less educated participants. As previously observed, neither gender nor hippocampal volumes showed any significant effect on NTMTB-time scores.

Finally, NBNT analyses presented quite particular results. NBNT scores were significantly influenced by the individual effects of APOE genotype ($F_{1,70} = 9.125$; $p = 0.002$), dichotomized age ($F_{1,70} = 4.867$; $p = 0.031$), dichotomized years of education ($F_{1,70} = 12.296$; $p = 0.001$), and gender ($F_{1,70} = 7.543$; $p = 0.008$), while no significant interaction effects were

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Table 4. Demographics and relevant characteristics of control-high (*n* = 52) and control-low (*n* = 22) groups

Variables	Control-high group	Control-low group
Age, years	69.65±4.76	72.05±5.46
MMSE	29.62±0.49	26.95±1.21
Males/females	18/34	9/13
Education, years	19.06±4.84	12.41±5.18
APOE4 noncarrier/carrier	37/15	8/14 (1 homozygote)
RH-ICV	0.00245±0.00035	0.00229±0.00036
LH-ICV	0.00243±0.00037	0.00232±0.00037

Data are presented as mean ± SD or *n*. MMSE, Mini-Mental State Examination; APOE, gene encoding for apolipoprotein E; APOE4 noncarrier, there is not any ε4 allele; APOE4 carrier, there is at least 1 ε4 allele; LH-ICV, left hippocampal volume/intracranial volume; RH-ICV, right hippocampal volume/intracranial volume.

detected. Mirroring NTMTB-time results, such findings indicated an additive rather than multiplicative relationship among variables. Therefore, NBNT scores were lower in older, less educated subjects, females, and APOE4 carriers. Table 3 summarizes GLM results for the whole sample.

APOE4 Effects in Control-High and Control-Low Groups

The relevant demographic information of control-high and control-low groups is displayed in Table 4. When these groups were compared, no significant differences in terms of gender ($\chi^2_1 = 0.264$; $p = 0.607$) and dichotomized age ($\chi^2_1 = 3.734$; $p = 0.06$) were detected, although a tendency to more advanced age was observed in the control-low group. However, as it might be expected considering previous results, APOE genotype ($\chi^2_1 = 7.852$; $p = 0.005$) and years of education ($\chi^2_1 = 5.762$; $p = 0.016$) were not homogeneously distributed, with proportionally more APOE4 noncarriers and more subjects with years of education above the median in the control-high group. The relevance of such heterogeneity, especially the one regarding APOE genotype distribution, will be discussed below.

When APOE genotype effects were evaluated in the control-high group, no significant individual or interaction effects were observed in the analyzed tests. When APOE genotype effects were assessed in the control-low group, a different scenario appeared. In this group, APOE genotype exerted a significant effect on MMSE ($t_{20} = 2.630$; $p = 0.016$), IR ($t_{20} = 2.072$; $p = 0.048$), DR ($t_{20} = -2.846$; $p = 0.010$), and NBNT ($t_{20} = 3.291$; $p = 0.004$). As previously observed in the whole group, APOE noncarriers exhibited significantly higher scores in all tests. Considering sample's composition, a straightforward interpretation of interaction effects was not possible (only 3 younger APOE4 carriers, for example). Nevertheless, it was noteworthy that NBNT was significantly influenced by dichotomized age ($t_{20} = 2.166$; $p = 0.043$) and years of education ($t_{20} = -2.652$; $p = 0.016$), while education exerted a nominally significant effect on IR ($t_{20} = -1.974$; $p = 0.06$). As previously described, older and less educated subjects exhibited lower scores.

Discussion

Several investigations demonstrated a deleterious effect of APOE4 on the human brain. Perhaps the more frequently cited example is the association between APOE4 and an enhanced amyloid deposition in the form of plaques that appears in AD [47, 48]. Some additional

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DOI: 10.1159/000481852

processes such as the regulation of oxidative stress and neurotransmitter release, and the myelin sheath building are influenced by APOE genotype as well [49–52]. Of special relevance is the implication of APOE in neural repair and plasticity (see [5]). An illustration of such involvement is the poorer recovery and worse memory performance of APOE4 carriers with traumatic brain injury [53], or the lower benefit after a multidomain cognitive training in those healthy elders who are APOE4 carriers [54]. This fact may be considered an indication that APOE4 not only influences cognitive status by merely increasing the risk of AD, but more broadly by exerting a negative influence on a variety of neurobiological processes. If this evidence is accepted, it seems reasonable to assume that APOE4 might produce a series of accumulative deficits on brain functioning leading to an impaired cognitive performance, irrespective of the subsequent development of AD. In fact, some studies reported cognitive deficits in healthy APOE4 carriers at remarkably young ages [7, 55], a greater acceleration of memory decline prior to age 60 [56], and a worse cognitive status in the ninth decade [57]. Overall, results of these investigations represent a strong support for the cognitive phenotype hypothesis and for the notion that APOE4 effects exceed the AD spectrum. Notwithstanding, that hypothesis has been challenged by some studies indicating that cognitive impairment in so-considered healthy population is produced by the inadvertent inclusion of preclinical or prodromal AD cases [42, 58–60]. Essentially, these studies defend the idea that APOE4's influence on cognitive performance is secondary to its capability to increase the risk of AD.

In this regard, our current results parallel a number of previous investigations [44, 61–63]. Healthy APOE4 carriers exhibited a significantly poorer performance in several cognitive domains, including global cognitive functioning, episodic memory, verbal fluency, and naming. This effect emerged when the whole sample (control-high + control-low groups) was analyzed, and was modulated by the influence of age and education, indicating a clear tendency to poorer cognitive performance in older and less educated APOE4 carriers. Mirroring the report of Foster et al. [44], when subjects were rigorously classified according to their performance on the MMSE, APOE4 effects vanished in the control-high group (MMSE score ≥29) and remained for global cognitive functioning and episodic memory in the control-low group (MMSE score <29). At first glance, these findings might represent a solid support for the preclinical/prodromal hypothesis of AD, but their actual significance depends, as in some previous representative studies that will be discussed, on the interpretation they receive. A good example of this key issue is the interpretation of the results of Winnock et al. [41]. They found a significant effect of APOE genotype on MMSE scores in a sample of nondemented subjects, but the effect disappeared after adjustment for education. Also, cognitive decline over time was independent of APOE genotype. These findings have been understood as further support for the preclinical/prodromal hypothesis (see [44]). However, Winnock et al. [41] reported a strong heterogeneity of the education level that was dependent on APOE genotype, since educational attainment was "much higher" in noncarriers. According to this, the authors suggested that APOE4 might play a direct role on cognition since early life by influencing the level of education that could be attained.

Undoubtedly, this is a challenging argument that reinforces the cognitive phenotype hypothesis. Our results showed similar and complementary tendencies. First, we detected a noticeable, though nonsignificant, trend to higher educational levels in the noncarriers group. Second, we found a significant interaction of APOE genotype and education that influenced global cognition, episodic memory, and language domains in the whole sample. Importantly, this interaction might provide valuable information on the role of education in cognitive aging. Thus, although more educated subjects tended to show increased cognitive scores, such trends were remarkably significant in APOE4 carriers. In this subsample, more educated carriers obtained significantly higher scores than less educated carriers, while the effects were not significant in noncarriers. Additionally, no significant differences in cognitive per-

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formance emerged when highly educated subjects were compared according to their APOE genotype. Such differences appeared as strongly significant (Fig. 2) when less educated subjects were compared, with APOE4 carriers showing a notably poorer performance than noncarriers. From our point of view, this evidence represents a support for an active, rather than "confounding," influence of CR. This finding is in agreement with classical [16, 18] and more recent reports [17] that demonstrated a positive correlation between mental status and educational level. It is well established that those subjects with a higher level of CR usually exhibit greater evidence of brain pathology before the appearance of clinical manifestations [64–66], and once they emerge, they exhibit a faster cognitive decline [67]. However, it should be pointed out that this effect is also observable in healthy elderly population, finding a negative correlation between CR and brain atrophy and a positive relationship between CR and cognitive performance [68–70]. With respect to the potentially active and protective role of education, Amieva et al. [71] accomplished a 20-year follow-up study of a population with MCI and evaluated the capability of education to delay a full-blown AD once first cognitive symptoms had appeared. Their results indicated that higher-educated individuals were able to "resist" an already existent decline during an 8-year period, thus supporting the notion of an active compensatory process associated with education. On the same line, a recent study performed by Vaqué-Alcázar et al. [72] suggested that education exerts a protective role against age impact in both brain and cognitive deterioration in healthy aging. Our findings pointed out a positive effect of education that also seemed to increase subjects' endurance when they are affected by a deleterious influence such as APOE4. Therefore, education impact may be exerted by means of the implementation of efficient cognitive strategies, thus indicating an active process that might prevent the onset of an evident deterioration [73].

Another important controversy in this field is related to age modulation of APOE4 effects. Bender and Raz [74] found than even young APOE4 carriers exhibited a higher vascular risk, poorer cognitive performance, and reduced prefrontal volumes, and this effect was more noticeable with age. The meta-analysis by Small et al. [62] revealed APOE4 influences on a series of cognitive domains that were also modulated by age. The authors reported that the magnitude of APOE4-related deficits was inversely associated with age, with fewer differences in older participants; though they admitted the effect was weak due to the small number of studies included. A later meta-analysis by Wisdom et al. [63] reported larger effect sizes than previously observed by Small et al. [62] but essentially in the same domains: episodic memory, global cognitive functioning, executive functioning, and perceptual skills. However, contradicting the study of Small et al. [62], the interaction with age showed an opposite tendency, with older APOE4 carriers exhibiting poorer performance. Similarly, Foster et al. [44] reported that cognitive performance was particularly impaired in older carriers. Our results supported such relationship between age and APOE4-related deficits. Although noncarriers showed an overall tendency to higher cognitive status than carriers, the effect was more evident in the older participants indicating that APOE4 effects seemed to interact with aging. Nevertheless, at this point the interpretation debate emerges again. The fact that healthy older carriers tend to show a more impaired cognition when compared with healthy younger carriers might be considered a new support for the phenotype hypothesis, which claims that APOE4's harming effects are accumulative over time, and consequently they should be more evident in the aged. Such notion also applies to the concept of antagonistic pleiotropy [75]. This is to say that a gene evolutionarily selected to offer certain advantages in the early stages of the life-span may produce detrimental effects when acting in later stages. For instance, Evans et al. [76] found that middle-aged APOE4 carriers performed as well as or even better than APOE4 noncarriers on different cognitive tasks, making this advantage reduced when compared with younger subjects. However, the recruitment of brain regions observed during the performance of these tasks in middle-aged APOE4 carriers was similar

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to that usually described in older individuals. In contrast, Foster et al. [44] claimed that age interaction with APOE genotype was only present when healthy controls and MCI cases were merged but disappeared once MCI cases were eliminated from the analyses. Accordingly, this might be understood as a new illustration of the notion that APOE4's main effect is to increase the risk of dementia rather than to produce a specific cognitive phenotype. Our results paralleled this observation, since the interaction between APOE genotype and age vanished once control-high and control-low groups were separated.

As it might be deduced from the preceding comments, the main aim of this discussion was to highlight the limitations and controversies of the current *state of the art* in this field of investigation. Perhaps the most critical issue in this controversy is to determine whether methodologies such as the one utilized by Foster et al. [44] that inspired our study or previous retrospective approaches allow an actual refutation of the cognitive phenotype hypothesis. In this vein, the study of Foster et al. [44] and ours coincided in a fundamental finding: the number of APOE4 carriers was disproportionately larger in the MCI group (Foster's study), and also in the group of healthy cases that may be more susceptible to present subtle cognitive deficits (our control-low group). Again, this might be considered as a clear indication that APOE4 effects on cognition are due to the biasing action of potential prodromal AD cases. Thus, the main conclusion of the present study is that our results might be considered a new support for the preclinical/prodromal hypothesis of APOE4 effects on cognition. However, an equally important claim is that our experimental approach and all previous approaches supporting this hypothesis were not sufficiently based on neuropathological data to discard the cognitive phenotype alternative. The only procedure that may allow an actual refutation should necessarily rely on the use of amyloid deposition markers to rule out the influence of prodromal or even preclinical AD cases. Consequently, this is a very important (see [77–79]) but not the only limitation of our study. The sample was relatively small, particularly the control-low group (see also the reduced number of younger APOE4 carriers), limiting the generalization of the present results. Second, we did not carry out a longitudinal study which, according to our perspective, could provide essential cues for the hypotheses discussed here. Assuming these limitations, our study still offers some particular strengths compared with previous publications in this field. Those strengths were due to the very strict classification procedure utilized, which surpassed the mere combination of healthy and cognitively impaired cases, and were due to the inclusion of factors such as hippocampal volumes, not previously considered. Forthcoming studies should address the role of the APOE genotype in aging from a multifactorial perspective, including biomarkers and a follow-up of the participants.

Acknowledgements

This study was supported by two projects from the Spanish Ministry of Economy and Competitiveness (PSI2009-14415-C03-01 and PSI2012-38375-C03-01), a postdoctoral fellowship to María Eugenia López (FJCI-2014-22730) from the Spanish Ministry of Economy and Competitiveness, and a predoctoral fellowship from the Spanish Ministry of Education, Culture and Sports (FPU13/02064) to Inmaculada Concepción Rodríguez-Rojo.

Disclosure Statement

The authors declare no competing financial interests.

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