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Featured Article

Exploring *APOE* genotype effects on Alzheimer's disease risk and amyloid β burden in individuals with subjective cognitive decline: The FundacioACE Healthy Brain Initiative (FACEHBI) study baseline results

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Abstract

Introduction: Subjective cognitive decline (SCD) has been proposed as a potential preclinical stage of Alzheimer's disease (AD). Nevertheless, the genetic and biomarker profiles of SCD individuals remain mostly unexplored.

Methods: We evaluated apolipoprotein E (*APOE*) ε4's effect in the risk of presenting SCD, using the Fundacio ACE Healthy Brain Initiative (FACEHBI) SCD cohort and Spanish controls, and performed a meta-analysis addressing the same question. We assessed the relationship between *APOE* dosage and brain amyloid burden in the FACEHBI SCD and Alzheimer's Disease Neuroimaging Initiative cohorts.

Results: Analysis of the FACEHBI cohort and the meta-analysis demonstrated SCD individuals presented higher allelic frequencies of *APOE* ε4 with respect to controls. *APOE* dosage explained 9% (FACEHBI cohort) and 11% (FACEHBI and Alzheimer's Disease Neuroimaging Initiative cohorts) of the variance of cerebral amyloid levels.

The authors have declared that no conflict of interest exists.

[1]Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pd.

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Discussion: The FACEHBI sample presents *APOE* ε4 enrichment, suggesting that a pool of AD patients is nested in our sample. Cerebral amyloid levels are partially explained by the *APOE* allele dosage, suggesting that other genetic or epigenetic factors are involved in this AD endophenotype.

Keywords: Subjective cognitive decline; Alzheimer's disease; *APOE* alleles; Amyloid burden; PET

1. Introduction

Neuropathological changes of Alzheimer's disease (AD) evolve several years before the onset of clinical symptoms [1,2]. Therefore, it is believed that characterization of earlier stages of AD, that is, mild cognitive impairment (MCI) and subjective cognitive decline (SCD), could beget new strategies to diagnose and treat the disease earlier [3].

An SCD population represents a subset of cognitively normal individuals with self-reported cognitive impairment [4]. It is suggested that a prodromal AD subgroup could be nested in an SCD population. Nevertheless, there is a scarcity of research studying both genetic and biomarker profiles of SCD individuals. New studies might help to improve the identification of those SCD individuals at risk of AD.

Thus far, presence of the apolipoprotein E (*APOE*) ε4 allele, the major genetic risk factor for AD [5], has been the only genetic marker associated with risk of SCD [6]. In a previous meta-analysis with 6824 individuals, we were able to estimate that *APOE* ε4 was significantly associated with risk of SCD [odds ratio [OR] = 1.15 (1.02–1.30); $P = .03$] [6]. Although the contribution of other genes has been explored, that is, *IL1B* or *TNF* [7], the risk of presenting an SCD diagnosis or of converting from SCD to AD has not been associated with other genetic signals [7,8].

Traditional AD-biomarker research has been focused on assessing cerebral amyloid aggregation and neuronal pathology, both of which are considered classical neuropathological hallmarks of AD [9]. It has been demonstrated that *APOE* alleles contribute to the biological modulation of amyloid β (Aβ) clearance [10]. Furthermore, genome-wide association studies (GWASs) evaluating the cerebral amyloid burden endophenotype have reinforced *APOE's* role in amyloid accumulation [11,12]. Despite the inextricable link between *APOE* and Aβ burden in AD patients, none of the reported endophenotype GWASs included SCD cohorts [11,12]. Thus, *APOE's* role in amyloid burden in the SCD population remains poorly examined.

Several studies have detected higher cerebral amyloid burden in AD and MCI patients compared with cognitively normal individuals [13–15] and also in AD compared with MCI patients [13], but studies evaluating amyloid pathology in SCD have provided inconsistent results [15,16]. However, when *APOE* genotypes are considered, more predictable results are observed, that is, SCD individuals who are *APOE* ε4 carriers have shown higher amyloid positron emission tomography (PET) uptake than SCD or healthy control ε4 noncarriers [17]. Understanding genetic and biomarker profiles, as well as their interaction, in SCD individuals, will allow integration of multiple variables influencing AD risk, that is, *APOE* status, age, and amyloid burden, and enhance the discrimination of subjects at risk of conversion to AD.

Here, we analyze *APOE* genotypes, baseline cerebral amyloid profile, and their relationship in the Fundacio ACE Healthy Brain Initiative (FACEHBI) cohort, which included 200 SCD individuals enrolled in a long-term longitudinal study of cognition, biomarkers, and lifestyle [18]. We evaluated *APOE* ε4's effect in the risk of presenting SCD using a case-control design including the FACEHBI SCD cohort and a cohort of Spanish population-based controls and afterward by performing a meta-analysis including studies addressing the same question. To explore whether SCD individuals who carry the *APOE* ε4 allele present increased risk of MCI or AD compared with noncarrier SCD individuals, we calculated ORs using Fundacio ACE SCD, MCI, and AD cohorts. To explore the relationship between *APOE* and brain amyloid burden, first we analyzed the FACEHBI data and then extended the analysis by including an independent cohort from the Alzheimer's Disease Neuroimaging Initiative (ADNI) study. Finally, we explored whether the effect of *APOE* dosage on brain amyloid burden was homogenous across clinical diagnoses (controls, SCD, MCI, and AD) using the FACEHBI and ADNI cohorts.

2. Methods

2.1. Subjects

With the objective to explore the effect of *APOE* ε4 in risk of presenting SCD, we used 200 SCD individuals recruited from the FACEHBI study and 3032 population-based controls recruited by a cross-sectional epidemiological survey, described below. In addition, we sought to estimate the risk conferred by this genotype in SCD population to have MCI or AD, using the FACEHBI sample, the ACE MCI cohort (1170 MCI patients), and the ACE AD cohort (2517 AD patients), all of them recruited by Fundacio ACE (Supplementary Fig. 1). To avoid population stratification, all individuals were selected to be of white Mediterranean ancestry with registered Spanish ancestors (for two generations).

2.1.1. The FACEHBI cohort

The FACEHBI cohort comprises 200 individuals diagnosed with SCD (mean age, 65.8 ± 7.1 years; 62.5%

women), which are embedded in a long-term observational study [18]. The sample has been obtained from two different sources: individuals referred by their physicians to our memory clinic for study of cognitive impairment and individuals who came to our institution through an Open House Initiative. SCD was defined as the coexistence of cognitive complaints and a score of ≥8 on MFE-30, the Spanish version of the Memory Failures in Everyday Life Questionnaire (20); Mini–Mental State Examination ≥ 27; clinical dementia rating (CDR) = 0; and performance on Fundació ACE Neuropsychological Battery [19] within the normal range for age and educational level. Further description of inclusion and exclusion criteria is provided by Rodriguez-Gomez et al [18]. All participants gave written consent, and the protocol was approved by the ethics committee of the Hospital Clinic i Provincial (Barcelona, Spain) (EudraCT: 2014-000798-38).

All subjects were screened at baseline for brain amyloidosis with Florbetaben [18F] radio tracer using PET (FBB-PET). PET images were acquired after administration of single slow intravenous bolus (6 sec/mL) of 300 Mbq of FBB (NeuraCeq), in a total volume of up to 10 mL, during 20 minutes.

Genomic DNA was obtained from 200 μL of human whole blood using commercial methods. High-resolution melting procedures were performed to determine *APOE* genotypes. Polymerase chain reactions (PCRs) were carried out in a final volume of 5 μL, using 11 ng of genomic DNA, 0.3 μM of each primer, and 2.65 μL of 2X SYBR Fast Master Mix (Kapa Biosystems). PCR conditions were a denaturation step at 95°C for 2 minutes, 33 cycles at 95°C for 10 seconds, and at 69°C for 30 seconds. Melting curves were 95°C for 15 seconds (ramping rate 5.5°C/second), 45°C for 15 seconds (ramping rate of 5.5°C/second), and 95°C for 15 seconds (ramping rate of 5.5°C/second). A fluorometric register was performed at one acquisition register per each degree Celsius. Melting peaks and genotype calls were obtained using the Eco Real-Time PCR system (Illumina).

2.1.2. The ACE MCI cohort

We included a sample of 1170 MCI patients (mean age, 75.9 ± 7 years; 64.5% women) recruited and assessed at the Fundació ACE Diagnostic Unit (Barcelona, Spain) between January 2006 and July 2013. A diagnosis of MCI was assigned according to Petersen criteria [20,21] and the classification of Lopez et al. [22,23]. All subjects had a CDR of 0.5 and were assessed using the Mini–Mental State Examination; the Hachinski Ischemia Scale; the Bipolar Depression Rating Scale; and the Neuropsychiatric Inventory Questionnaire. DNA was extracted using standard procedures, and conventional real-time PCR procedures (Applied Biosystems) were used to obtain *APOE* genotypes. See Supplementary Material from Lacour et al [24]. for detailed information.

2.1.3. The ACE AD cohort

We included 2517 AD cases (mean age, 81.6 ± 16.3 years, 70.9% women), who were referred for evaluation of cognitive impairment by their primary care physicians or primary care neurologists. Diagnosis of dementia and type of dementia are established by consensus according to DSM-IV criteria for dementia and NINCDS-ADRDA criteria for possible or probable AD. Further information of inclusion criteria and genotyping procedures is provided by Seshadri et al [25] and Boada et al [26].

2.1.4. Population-based controls

We used 3032 Spanish controls (mean age 54, ±11.7 years, 61.8% women) with unknown cognitive status recruited from a cross-sectional population-based epidemiological survey to describe the prevalence of cardiovascular risk factors in the general population, previously described [27,28]. Survey procedures were adapted from the World Health Organization MONICA Project (WHO MONICA) protocol. DNA extraction and *APOE* genotyping procedures were previously described by Seshadri et al [25].

2.1.5. The ADNI series

To validate our correlation analysis between *APOE* genotypes and brain amyloid in the FACEHBI cohort in an independent data set, we used 182 healthy controls (HCs), 103 SCD, 460 MCI, and 144 AD participants with available amyloid PET and *APOE* data from the ADNI study (http://adni.loni.usc.edu) [29]. Informed consent was obtained according to the Declaration of Helsinki. The ADNI PET core processes Florbetapir [18F] (AV45) PET images according to previously described methods [30].

2.2. Statistical analysis and meta-analysis

2.2.1. Risk analysis of the APOE locus in SCD

To investigate the effect of carrying an *APOE* ε4 allele in the risk of presenting SCD, we performed allelic frequency comparisons with a χ^2 test between 200 SCD individuals and 3032 Spanish population-based controls. Similarly, to explore whether SCD individuals who are carriers of *APOE* ε4 present increased risk of MCI or AD, we calculated ORs using Fundacio ACE SCD, MCI, and AD cohorts. Logistic regression analysis (additive model) was used to perform adjustments per (1) gender and (2) gender and age. All statistical analyses were performed using PLINK 1.9 software (http://www.cog-genomics.org/plink2) [31].

In addition, meta-analysis techniques were used to estimate the *APOE* ε4's effect in the risk of presenting SCD. We have updated a previous meta-analysis conducted by our group, which included studies published before 2015 and a total of 6824 individuals [6], with studies published before July 2017. Literature search in PubMed was performed using *APOE* and SCD terms. We selected the studies meeting the following criteria: (1) case/control studies or longitudinal studies, where it is possible to distinguish a subpopulation of SCD individuals and a subpopulation of

healthy controls; (2) studies that provide a complete definition of the participants; and (3) studies that provided an OR with 95% confidence interval (CI) as well as the *P*-value or provide sufficient data to calculate them. Studies with overlapping samples were excluded. After the incorporation of data from the FACEHBI cohort, a total of 12,183 individuals were included (Supplementary Fig. 2). SCD definition and recruitment strategy for each included study have been detailed in Supplementary Table 1. Effects were determined using the inverse variant method (fixed-effects model). In the case of heterogeneity, the DerSimonian and Laird method (random-effects model) was used. Heterogeneity was considered significant when $I^2 > 50\%$ and $P < .05$. Pooled effects and forest and funnel plots were obtained using Metafor package from R.

2.2.2. *Effect of the* APOE *locus on brain amyloid burden*

We used baseline individual standardized uptake value ratios (SUVRs) from the FACEHBI and ADNI. First, we tested for normality of FBB and AV45 global SUVR measures and found they were not normally distributed. Thus, we decided to log-transform the data to conduct the consecutive analyses. *APOE* genotypes were codified according to the ε4 allele dosage, that is, ε4ε4 = 2; ε3ε4 = 1; ε3ε3 = 0; ε2ε4 = 0; ε2ε3 = −1; ε2ε2 = −2. The present analyses were carried out using SPSS 20.0 (SPSS Statistics 20; IBM Corporation, Somers, NY, USA).

2.2.2.1. *Global SUVR according to* APOE *dosage in the FACEHBI cohort*

We performed an analysis of variance to look for differences in global SUVR across *APOE* dosage (five groups) in the FACEHBI cohort. Post hoc analysis, including Bonferroni correction for multiple comparisons, was performed to check differences between groups. *APOE* ε2ε2 genotype was not considered in post hoc testing because of its small sample size (n = 1). Ggplot2 package from R was used to depict differences between groups.

2.2.2.2. *The relationship between* APOE *dosage and brain amyloid burden*

We sought to estimate the relationship between *APOE* ε4 allele dosage and brain amyloid burden in the FACEHBI SCD cohort and in an independent cohort from the ADNI. The ADNI cohort included individuals with different clinical diagnoses (HC = 182, SCD = 103, early MCI = 303, late MCI = 157, and AD = 144) to explore whether the relationship changed across different clinical categories. Because FACEHBI and ADNI use different radioactive tracers, we decided to standardize log-transformed amyloid SUVR to be able to compare results. We used linear regression model with SUVR as the dependent variable and *APOE* dosage as the independent variable, to check this correlation. Ggplot2 package from R was used to depict the individual slopes per status. Finally, two additional methods were used to explore whether there was a homogeneous correlation across differential clinical categories: (1) meta-analysis of the effect of *APOE* ε4 on brain amyloid burden across different clinical diagnoses; criterions for selection of the meta-analyses model was described in Section 2.2.1. Correlation coefficients were pooled using Meta packages from R and (2) the evaluation of an interaction term using the general linear model across the entire sample (n = 1089). Clinical categories were coded as follows: HC = 0; SCD = 1; early MCI = 2; late MCI = 3; and AD = 4.

We examined the relationship between age and brain amyloid burden using the same analyses described for *APOE* dosage. Age at baseline was used for the present analysis.

3. Results

3.1. *Risk analysis of* APOE *locus in SCD*

APOE markers (rs7412 and rs429358) followed the Hardy Weinberg equilibrium. Enrichment in the allelic frequencies of *APOE* ε2 and ε4 was detected in the FACEHBI sample with respect to the control population. Genotypic and

Table 1
Adjusted and unadjusted effects of *APOE* ε4 in the risk of SCD diagnosis and risk to have MCI or AD from SCD status

	%Genotype carriers (n)		Unadjusted		Adjusted per gender		Adjusted per gender and age	
	APOE ε4	*APOE* ε2	*APOE* ε4 OR (95% CI) *P* value	*APOE* ε2 OR (95% CI) *P* value	*APOE* ε4 OR (95% CI) *P* value	*APOE* ε2 OR (95% CI) *P* value	*APOE* ε4 OR (95% CI) *P* value	*APOE* ε2 OR (95% CI) *P* value
HC–SCD	18.4 (557)–26 (52)	12.1 (396)–14.5 (29)	1.61 (1.21–2.16) .001	1.20 (0.82–1.77) .34	1.61 (1.20–2.14) .0012	1.19 (0.82–1.75) .35	1.62 (1.20–2.19) .0016	1.31 (0.76–1.67) .54
SCD–MCI	26 (52)–32.4 (379)	14.5 (29)–8.1(94)	1.28 (0.95–1.72) .098	0.53 (0.34–0.82) .003	1.26 (0.95–1.69) .11	0.53 (0.35–0.82) .004	1.44 (1.05–1.97) .02	0.82 (0.49–1.40) .47
SCD–AD	26 (52)–44.7 (1125)	14.5 (29)–6.4 (63)	1.95 (1.47–2.60) 2.69e−06	0.42 (0.28–0.63) 1.90e−05	1.98 (1.49–2.64) 2.69e−06	0.44 (0.30–0.66) 7.38e−05	2.2 (1.59–3.05) 2.28e−06	0.53 (0.31–0.91) .02

Abbreviations: AD, Alzheimer's disease; *APOE*, apolipoprotein E; CI, confidence interval; HC, healthy control; MCI, mild cognitive impairment; OR, odds ratio; SCD, subjective cognitive decline.

allelic frequencies for the studied cohorts are reported in Supplementary Table 2.

Higher risk of having SCD was identified for HC *APOE* ε4 carriers with respect to noncarriers [OR = 1.61 (1.21–2.16); $P = .001$]. In the case of *APOE* ε2, a nonsignificant risk effect was detected [OR = 1.20 (0.82–1.77); $P = .34$] (Table 1). Similarly, when the comparison was conducted between SCD individuals and MCI patients, the risk of MCI was not significantly increased in SCD subjects carriers of *APOE* ε4 compared with noncarriers although a risk trend was observed [OR = 1.28 (0.95–1.72); $P = .098$] and a protective association was detected for *APOE* ε2 [OR = 0.53 (0.34–0.82); $P = .003$]. In the case of SCD individuals and AD patients, SCD individuals who are carriers of *APOE* ε4 present an increased risk of AD [OR = 1.95 (1.47–2.60); $P = 2.69e\text{-}6$], in contrast to *APOE* ε2's effect [OR = 0.42 (0.28–0.63); $P = 1.90e{-}5$] (Table 1). No major difference in the effect size of *APOE* ε4 and *APOE* ε2 was detected after adjusting for gender. After adjusting for gender and age, the effect for *APOE* ε2 in MCI and AD groups decreased and the significance level increased (Table 1). This effect was expected taking into account that age is an independent risk factor for AD and the presence of major differences in age among the studied cohorts.

The meta-analysis (n = 12,183) evaluating *APOE*'s effect in the risk of presenting SCD showed increased risk of SCD in HC carriers of the *APOE* ε4 allele [1.18 (1.06–1.31); $P = .002$] (Fig. 1). *APOE* ε4's effect show nonsignificant heterogeneity between studies ($I^2 = 39\%$, $P = .06$). Funnel plot is shown in Supplementary Fig. 3. Egger test for funnel plot asymmetry presented a P value = .99, supporting that publication bias is not present in our meta-analysis. Subpopulation analysis only for Caucasians [1.17 (1.05–1.29); $P = .004$] ($I^2 = 42\%$, $P = .06$) showed that the pooled effect was not modified when additional population was introduced.

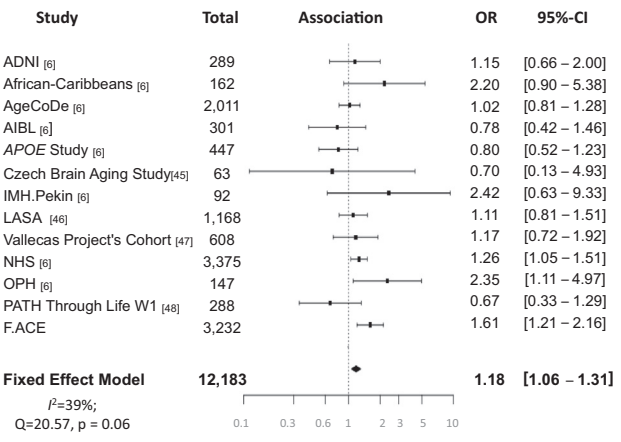

Fig. 1. Forest plot for the effect of *APOE* ε4 genotype in risk to be diagnosed with SCD. Abbreviations: ADNI, Alzheimer's Disease Neuroimaging Initiative; *APOE*, apolipoprotein E; CI, confidence interval; OR, odds ratio; SCD, subjective cognitive decline. For further information about Czech brain aging study, LASA, Vallecas Study, and PATH Study, see references [45], [46], [47] and [48], respectively.

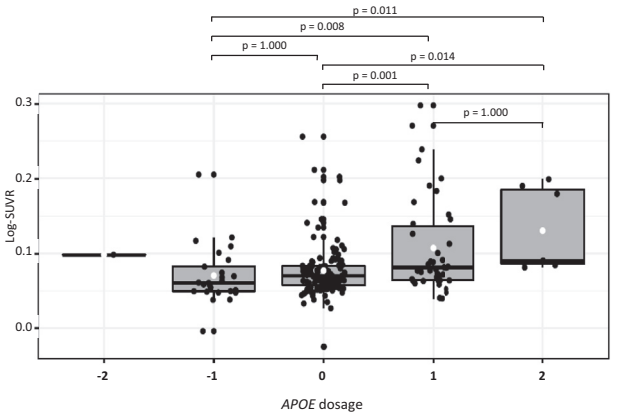

Fig. 2. Effect of *APOE* allele dosage in cerebral amyloid burden for the FACEHBI cohort. Mean is represented in white. Abbreviations: *APOE*, apolipoprotein E; SUVR, standardized uptake value ratio.

3.2. *Effect of* APOE *locus on brain amyloid burden*

3.2.1. *Global SUVR according to* APOE *dosage in the FACEHBI cohort*

Global SUVR was significantly different across *APOE* dosage groups in the FACEHBI cohort. Post hoc analysis revealed that those groups comprising *APOE* ε4 carriers were driving this difference (Fig. 2).

3.2.2. *The relationship between* APOE *dosage and brain amyloid burden*

APOE ε4 dosage explained 9% of the variance in brain amyloid burden ($R^2 = 0.09$; $P = 1.70e{-}5$) in the FACEHBI cohort (Table 2). When we performed the model taking into account *APOE* allele dosage and age, 15% of the variance was explained ($R^2 = 0.15$; $P = 5.25e{-}8$). *APOE* ε4 dosage explained 12% of the variance ($R^2 = 0.12$; $P = .004$) in the SCD ADNI cohort, which is in accordance with our findings (Table 2). Next, we investigated the relationship between amyloid accumulation and *APOE* allele dosage across the entire ADNI series, comprising HC, SCD, MCI, and AD cohorts. The highest correlation was detected in late-onset MCI individuals ($R^2 = 0.18$; $P = 2.86e{-}8$). Moreover, we observed an upward trend in the coefficient of determination from HC to late-onset MCI. However, the correlation experimented a decrease when the analysis was performed in AD individuals ($R^2 = 0.09$; $P = 2.00e{-}4$) (Table 2). Individual slopes per clinical categories are shown in Supplementary Fig. 4.

The meta-analysis showed that the correlation between *APOE* dosage and amyloid burden was not significantly heterogeneous across clinical groups ($I^2 = 7\%$; $P = .372$) (Fig. 3), and *APOE* ε4 allele dosage explained 11% of the variance in amyloid SUVR [R (95% CI) = 0.33 (0.28–0.40), $P < .001$; Fig. 3]. Although a significant interaction between the effect of *APOE* dosage and clinical status across the pooled data was detected ($R^2 = 0.27$; $P = .02$), the explained variance experimented minor changes when

Table 2
Determination coefficient and level of significance between *APOE* allele dosage and cerebral amyloid burden

Study	ADNI	FACEHBI	ADNI	ADNI	ADNI	ADNI
Status	HC	SCD	SMC	EMCI	LMCI	AD
N	182	200	103	303	157	144
Age (mean ± SD)	73.4 ± 6.3	65.8 ± 7.1	72.2 ± 5.6	71.3 ± 7.4	72.2 ± 7.5	74.4 ± 8.1
APOE ε4 carriers % (n)	28.6 (52)	26.0 (52)	31.1 (32)	43.2 (131)	57.9 (91)	67.4 (97)
R^2; *P* value	0.05; .002	0.09; 1.70e−5	0.12; 0.004	0.14; 1.95e−11	0.18; 2.86e−8	0.09; 2.00e−4

Abbreviations: AD, Alzheimer's disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; *APOE*, apolipoprotein; EMCI, early mild cognitive impairment; FACEHBI, Fundacio ACE Healthy Brain Initiative; HC, healthy control; LMCI, late mild cognitive impairment; PET, positron emission tomography; SCD, subjective cognitive decline; SMC, subjective memory impairment; SUVR, standardized uptake value ratio.

NOTE. SUVR data used for the present analyses were obtained after the standardization of log-transformed SUVR.

the model was run without considering the interaction factor (R^2 = 0.26).

Finally, we decided to explore the effect of age on amyloid burden. The meta-analysis of the correlation coefficient between age and amyloid burden detected high heterogeneity (I^2 = 82.7%; P < .0001) (Fig. 3). In addition, advanced age was not correlated with higher cerebral amyloid accumulation [R (95% CI) = 0.11 (−0.03 to 0.26); P = .13; Fig. 3]. A nonsignificant interaction term was observed between age and clinical status for

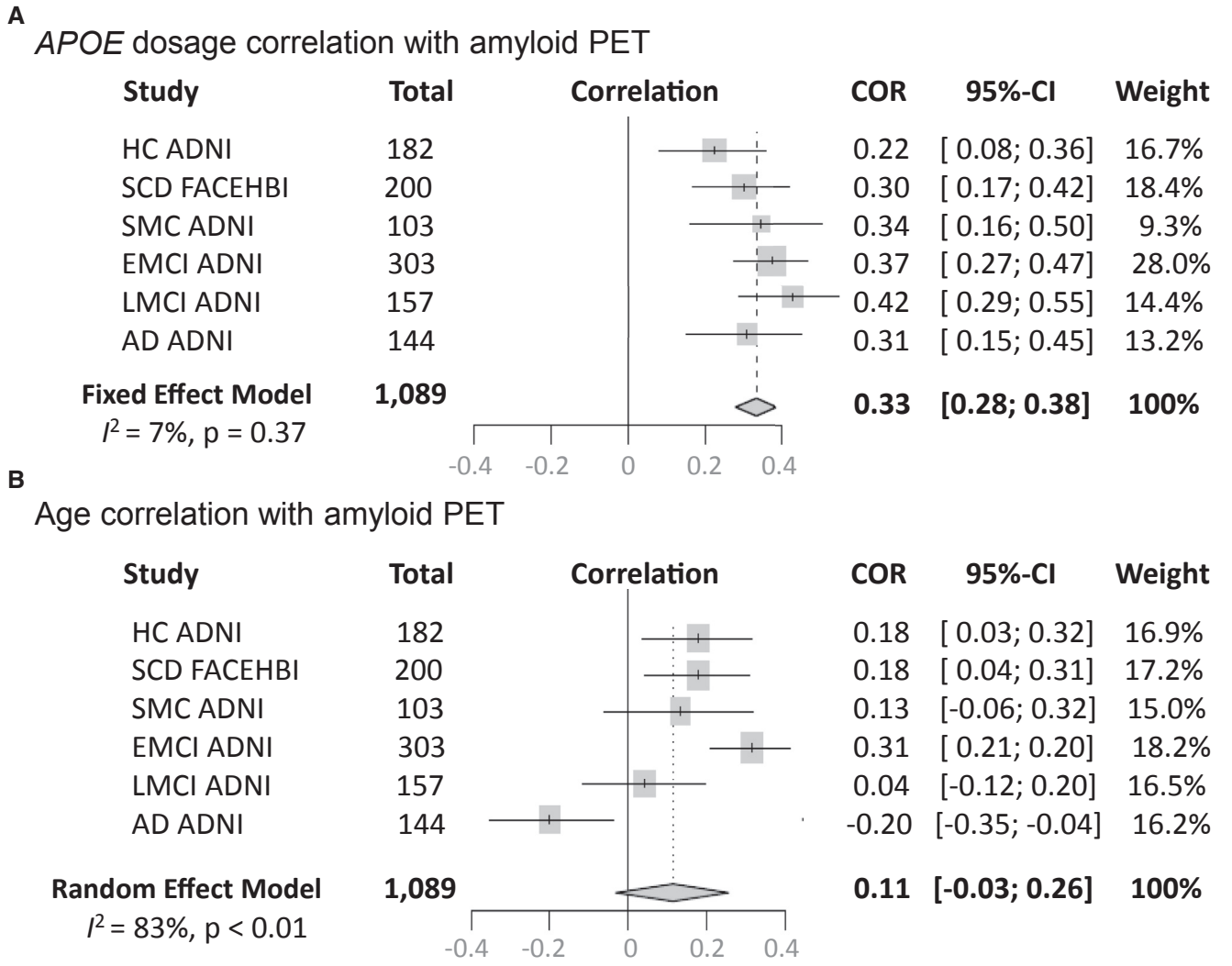

Fig. 3. Forest plot for (A) the effect of *APOE* ε4 and (B) the age, in amyloid burden across clinical categories. Abbreviations: AD, Alzheimer's disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; *APOE*, apolipoprotein; EMCI, early mild cognitive impairment; FACEHBI, Fundacio ACE Healthy Brain Initiative; HC, healthy control; LMCI, late mild cognitive impairment; PET, positron emission tomography; SCD, subjective cognitive decline; SMC, subjective memory impairment.

predicting variance in brain amyloid levels (R^2 = 0.19; P = .18). The proportion of variance explained when the model did not consider the interaction was 17% (R^2 = 0.17).

4. Discussion

SCD has been identified as a risk factor to have AD [32] and has been suggested as a potential preclinical stage of the disease [33]. Despite this, the genetic and biomarker profiles of SCD individuals remain mostly unexplored.

In this study, we report increased risk of SCD from healthy status in carriers of the *APOE* ε4 allele using the FACEHBI SCD sample, which supports that a pool of AD patients are nested in this cohort. We performed a large meta-analysis that demonstrated *APOE* ε4 is a genetic risk factor of presenting SCD. Furthermore, *APOE* ε4 carriers with SCD presented a significantly increased risk of AD diagnosis. The finding of *APOE* ε2 allele enrichment in the FACEHBI cohort (not observed in other data sets, i.e., ADNI) [OR (CI 95%) = 0.53 (0.37–0.74); P = 2.30e−4] was unexpected taking into account the protective role of *APOE* ε2 in AD [34]. There could be two reasons for this. Higher frequency of *APOE* ε2 allele has been detected in individuals with white matter hyperintensities [35,36]. Taking into account that there is an increment in white matter hyperintensities in subjects with vascular dementia [37], the enrichment of *APOE* ε2 in the FACEHBI population could be underlying a pool of subjects with brain vascular alterations. Random chance could also be underlying this variation in ε2 allelic frequency.

The SCD cohorts used in this meta-analysis present a certain level of heterogeneity (I^2 = 39%; P = .06), which could be due to differences in the selection of SCD and healthy controls individuals among studies. SCD comprises individuals who will convert to AD, individuals who will convert to other dementias, and others who will remain healthy. Moreover, other factors could be leading to this heterogeneity, that is, differences in recruitment strategies, assessment [38], and SCD definitions across studies [39], which researchers must carefully control for and standardize. In this sense, significant efforts are underway to design neuropsychological tools sensitive to subtle cognitive changes and to identify genetic and biomarker profiles in SCD subjects. The integration of genetic, neuropsychological, and biomarker profiles seems necessary to characterize SCD individuals and improve reproducibility of studies.

In an effort to improve characterization of SCD individuals, we combined *APOE* and amyloid burden information. We detected statistically significant differences in cerebral amyloid burden between *APOE* allele dosage stratums in the FACEHBI sample, which is in accordance with previous findings [40,41]. The variation in brain amyloid levels is partially explained by *APOE* genotype in our series. This finding was replicated in the ADNI data set by us and others [11].

The contribution of other genetic factors to cortical Aβ levels remains mostly unexplored, and a large proportion of the variance remains unexplained. Some studies describe models that consider the contribution of other genetic markers, that is, *APOE* ε4 and *BCHE*-rs509208 explained 15% of the variance [11] and *IL1RAP*-rs12053868 explained 7.1% of the variance [12]. However, when the model considered the polygenic risk score of genes identified in the stage I of the International Genomics of Alzheimer's Project study, only 1% of the variance was explained [42]. The involvement of nonamyloid processes in AD could be explaining this inconsistency. Either way, it seems necessary to include the genetic markers associated with the amyloid cascade in the model. The finding of substantial overlap between genes associated to AD pathology with those driving cerebral amyloid burden would support the use of this trait as an AD endophenotype.

After exploring the correlation between *APOE* and amyloid burden across clinical categories, a decrease in the correlation coefficient was observed in the AD group. This result could be explained by the effect of atrophy in amyloid PET measurements. Some works have proposed partial-volume effect can be distorting PET signal and diminishing the accuracy of the measure [43]. On the other hand, a subtle interaction effect was detected between *APOE* and clinical categories predicting amyloid burden. This interaction was not detected using the meta-analysis strategy, maybe, because it might be underpowered. In that scenario, adjusting for clinical category is mandatory when individuals with different clinical status are merged for studying the brain amyloid burden endophenotype with GWAS methodology.

In the same way, we also evaluated the role of age in determining the Aβ brain burden endophenotype across the AD continuum. Age has been suggested as a nongenetic risk factor for AD [44], and several studies have pointed to it as a risk factor for cerebral amyloid burden [15]. Conversely, we did not detect a correlation between age and Aβ load, and an inverse correlation was detected in the AD group, which can be caused by partial-volume effect. Older individuals might present an advanced stage of the disease, showing greater atrophy that could be disturbing PET measurements. In addition, an interaction effect was not detected between age and clinical category predicting the change of amyloid burden, which is not unexpected considering that age did not modulate this endophenotype. AD is related with both advanced age [44] and the presence of neuritic plaques [9]. This dual association can generate a confounding relation when age and Aβ are analyzed independently from AD. Hence, we propose that age acts as a confounding factor, which is not directly associated with the Aβ endophenotype.

There are potential limitations in the present study. We were not able to genotype *APOE* alleles at the same moment for different cohorts. Despite that, we check that both *APOE* markers (rs7412 and rs429358) were following Hardy Weinberg expectations for each comparison. In addition, to evaluate the correlation between *APOE* dosage and amyloid burden, we used data from the FACEHBI and ADNI samples, which use different radioactive tracers for amyloid PET scans. In an effort to control for discrepancies between studies, we standardized the log-transformed SUVR measure. Finally, the present correlation analyses were not adjusted by time of disease duration, which could be an interesting point for future studies. Despite that, taking into account that analyses were conducted separately per clinical stratum, major differences are not expected.

In summary, the present data support the role of *APOE* ε4 as a risk factor of SCD and its involvement in brain amyloid burden in SCD subjects. Amyloid-PET is an instrumental measure of the brain amyloid burden endophenotype in SCD subjects, but the modeling of this important trait will require further integration of other genetic and epigenetic factors.

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Supplementary data

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RESEARCH IN CONTEXT

1. Systematic review: Subjective cognitive decline (SCD) individuals are at risk of having Alzheimer's disease (AD). Despite this, there is a scarcity of research studying genetic and biomarker profiles of the SCD population. A comprehensive pubmed search of previous publications analyzing *APOE* effect on SCD subjects was conducted.
2. Interpretation: We evaluated *APOE* ε4's effect in the risk of presenting SCD and analyzed the relationship between *APOE* ε4 and cerebral amyloid burden in SCD using data from the FACEHBI study, one of the largest single-site SCD cohorts with longitudinal amyloid PET and cognitive data, and from other independent SCD and AD cohorts. We detected enrichment of *APOE* ε4 in the FACEHBI cohort, suggesting that a pool of AD patients are nested in this sample and confirmed *APOE* ε4 is a risk factor of presenting SCD.
3. Future directions: We found that *APOE* ε4 only explains 10% of brain amyloid burden variability in SCD suggesting that other genetic or epigenetic factors are involved in this AD endophenotype. Furthermore, the variable relationship between *APOE* and brain amyloid burden across clinical diagnoses highlights the importance of adjusting for clinical status when conducting future genome-wide association studies of cerebral Aβ levels.

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