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*APOE*ε2 is associated with milder clinical and pathological Alzheimer's disease

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Abstract

Objective—The Alzheimer disease (AD) *APOE*ε4 risk allele associates with an earlier age of onset and increased amyloid-β deposition, whereas the protective *APOE*ε2 allele delays the onset and appears to prevent amyloid-β deposition. Yet the clinical and pathological effects of *APOE*ε2 remain uncertain because of its relative rarity. We investigated the effects of *APOE* ε2 and ε4 alleles on AD pathology and cognition in a large US dataset of well characterized AD patients.

Methods—We studied individuals from the National Alzheimer's Coordinating Center (NACC) autopsy cohort across the entire clinico-pathological continuum of AD. Multivariable models were built to examine the associations between *APOE* alleles and AD neuropathological changes, using the *APOE*ε3/ε3 group as comparator. Mediation analysis was used to estimate the direct and indirect effects of *APOE* alleles on AD pathology and cognition (CDR-SOB and MMSE).

Results—Compared to *APOE*ε3/ε3, *APOE*ε2 is independently associated with lower Braak NFT stages and, possibly, fewer neuritic plaques, but has no direct effect on CAA severity, whereas *APOE*ε4 is associated with more neuritic plaques and CAA, but has no independent effect on Braak NFT stage. Unadjusted analyses showed marked differences among *APOE* genotypes with respect to cognitive performance (ε2>ε3>ε4). Mediation analysis suggests that this is largely explained through effects on pathology.

Interpretation—Even when adjusted for age of onset, symptom duration and other demographic variables, *APOE*ε2 is associated with milder AD pathology and less severe antemortem cognitive

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impairment compared to *APOE* ε3 and ε4 alleles, suggesting a relative neuroprotective effect of *APOE*ε2 in AD.

Keywords

Alzheimer disease; amyloid plaques; apolipoprotein E; cerebral amyloid angiopathy; neurofibrillary tangles

Introduction

The association of the apolipoprotein E (*APOE*) gene with sporadic Alzheimer disease (AD) is the strongest of any known risk gene. Compared with the major ε3 allele, the *APOE*ε4 allele increases the risk of AD in a dose-dependent but not proportional fashion: carrying one copy of the ε4 allele confers a 3-4 times increased life-risk of developing AD, whereas carrying two copies increases this risk 8-12 times[1–4]. By contrast, compared to the major *APOE*ε3 allele, the *APOE*ε2 allele reduces the risk of developing AD by almost half[5–9].

A number of postmortem quantitative studies have established that the *APOE*ε4 allele correlates with increased amyloid plaque deposition in the brain[3,10–20] and with increased vascular amyloid deposition in the form of cerebral amyloid angiopathy (CAA)[10,20–26]. These findings have been more recently corroborated by *in vivo* amyloid PET imaging not only in AD patients, but also in cognitively intact elderly people[27–31]. While *APOE*ε3/ε3 carriers can still develop AD, taken together, this evidence emphasizes the role of *APOE*ε4 as an initiator of the amyloid cascade that ultimately leads to fully established AD [32,33]. Conversely, although some postmortem studies[14,16,17,19,34] have ascribed increased numbers of neurofibrillary tangles (NFTs) —the other pathological hallmark of the disease— to the *APOE*ε4 allele, others have not found such association[11,20], including those controlling for plaque burden in multivariable models[35,36].

In contrast to *APOE*ε4 carriers, *APOE*ε2 carriers are thought to bear fewer amyloid plaques[14,17,19,34,37,38] and possibly also fewer NFTs[14,34]. However, in part because of the relative rarity of this allele, sufficiently powered and detailed postmortem studies examining the effects of the *APOE*ε2 allele on the pathobiology of AD are still lacking.

We examined the association between the *APOE* alleles and the AD pathological hallmarks and cognitive outcomes using the National Alzheimer's Coordinating Center (NACC) autopsy cohort, a large multicenter longitudinal cohort study of aging involving the 34 past and present National Institute on Aging-funded Alzheimer Disease Centers (NIA-funded ADCs) across the United States. We hypothesized that the large size of this multi-center intensively studied cohort would enable us to find novel pathophysiological relationships that may have remained undetectable in prior studies due to lack of statistical power. Because concurrent Lewy bodies and cerebrovascular pathologies have been reported to contribute to cognitive impairment in prior population-based clinico-pathological studies, in order to examine the impact of *APOE* alleles on AD-related pathological and cognitive outcomes, we selected individuals with no primary neuropathological diagnosis other than AD neuropathological changes. Specifically, we aimed to evaluate the effects of the ε2 and ε4 alleles on the main pathological hallmarks of AD [neuritic plaques, neurofibrillary

tangles (NFTs), and cerebral amyloid angiopathy (CAA)]; and assess if there is a direct or indirect effect of these *APOE* alleles on the severity of cognitive impairment using mediation analysis.

Subjects & Methods

Inclusion and exclusion criteria

Subjects were participants in a longitudinal cohort study of aging in any of the past and present 34 NIA-funded ADCs. This multicenter study has been described in detail elsewhere[39–41]. Briefly, subjects undergo a baseline visit and annual follow-up visits in which a Uniform Data Set (UDS) is completed, including a minimum subject demographics data set, and standard motor, behavioral, functional, and neuropsychological assessments. Subjects were eligible for this study if they met the following inclusion criteria: 1) no primary neuropathological diagnosis other than AD neuropathological changes was found at autopsy; 2) final clinical evaluation within 2 years of death; 3) age of death 50 years old or older; and 4) *APOE* genotype available. Exclusion criteria were aimed at minimizing statistical noise in genotype-phenotype correlations and included: 1) a primary neuropathological diagnosis other than AD neuropathological changes (i.e., frontotemporal lobar degeneration, dementia with Lewy bodies, hippocampal sclerosis, vascular dementia, prion disease, Parkinson disease, Huntington disease, hypoxia, ischemia, necrosis, hemorrhage, other non-neurodegenerative diagnosis); 2) cognitive impairment attributable to alcohol use, depression, medication use, or medical illness; 3) carrying an *APOE*ε2/ε4 genotype (since we aimed at investigating the separate effects of *APOE*ε2 and *APOE*ε4 on AD pathological lesions and cognitive outcomes), and 4) being cognitively intact (CDR-SOB=0) at last clinical evaluation (in order to prevent artificially significant results due to the “anchoring” effect of cognitively intact subjects, who typically have low levels of AD pathology and are less likely to carry the *APOE*ε4 allele).

Data collection

Demographic and clinical data used in this study included sex, years of education, age of death, age of onset, symptom duration (age of death – age of onset), *APOE* genotype, last clinical dementia rating sum of boxes (CDR-SOB)[42] score, and last Mini Mental State Examination (MMSE)[43] score. The education level was categorized in 4-year intervals roughly corresponding to the education stages of high school, undergraduate college, and post-college education. Since we excluded subjects with CDR-SOB=0, values of CDR-SOB in this study range from 0.5 to 18, with higher values indicating worse cognitive/functional status. Supplemental tables 1 and 2 provide information about cognitively intact (CDR-SOB=0) subjects for comparison purposes.

Neuropathological variables included the Braak stage of NFTs (0: none; I-II: entorhinal; III-IV: limbic, and V-VI: isocortical)[44,45], the CERAD score of neuritic plaques (none/sparse, moderate, or frequent)[46], the presence of incidental Lewy bodies in any region, and the extent of vascular pathology (CAA, small and large vessel disease, and hippocampal sclerosis). Although the Thal phases of amyloid deposition[47] have been recently implemented in NACC neuropathological assessment, they were not available for most

subjects in this study, therefore we could not use the ABC score of AD neuropathological changes[48,49] for clinico-pathologic correlations. While more objective and quantitative methods of assessment are under development, NACC neuropathology guidelines recommend the use of a qualitative and subjective grading system of the overall severity (rather than an individual vessel) to assess arteriosclerosis, atherosclerosis, and CAA (none, mild, moderate, or severe). Arteriosclerosis refers to the hyalinosis of the media and adventitia (arteriolosclerosis) of small parenchymal and/or leptomeningeal vessels, whereas atherosclerosis refers to the presence of intimal and medial fibro-fatty atheromatous plaques in large arteries at the base of the brain (i.e., circle of Willis). Hippocampal sclerosis was defined as the presence of selective neuronal loss and gliosis ("sclerosis") limited to CA1 and subiculum, with variable additional involvement of endplate, CA2, entorhinal cortex, and amygdala.

Statistical analyses

The association of the presence of *APOE* alleles with ordered categories of AD pathological hallmarks (neuritic plaques, NFTs, or CAA) in the cognitively impaired (CDR-SOB>0) group was examined with adjacent-categories logit models[50], which allow a covariate to have a constant effect across some or all categories, using the "VGAM" package in R software[51]. This flexibility of the adjacent-categories logit model enabled us to fit a parsimonious regression model for ordered categories of AD pathological hallmarks without strong parametric assumptions on the relationship among ordered categories. For each covariate, the assumption that it has the same effect for each category was checked using likelihood ratio tests of nested models. Whenever the assumption was violated, nested sequences of weaker assumptions permitting separate effects of a covariate for some logits were considered and were checked using likelihood ratio tests, until the most parsimonious assumption allowing different effects for different logits was not rejected. We have previously used this approach to examine the correlations between neuropathology and cognition (CDR-SOB) across the AD continuum[52].

To assess the possible pathobiological pathways linking *APOE* alleles with AD pathological hallmarks and whether there is a direct effect of each of these alleles on cognitive performance and/or an indirect effect through any of the AD pathological hallmarks, mediation analysis was conducted using the counterfactual framework[53]. To adjust for multiple mediators simultaneously, the methods proposed by Imai and Yamamoto[54] were adopted. Because neuritic plaques and NFTs from different *APOE* genotypes are biochemically and immunohistochemically similar, we assumed that no interaction exists between *APOE* genotype and the AD pathological hallmarks with respect to cognitive performance; in other words, that the effect of the pathological mediators on cognition does not differ across *APOE* genotypes for a given severity score or stage of pathology (i.e., Braak NFT stage VI). We made this same assumption of no interaction between *APOE* genotype and each AD pathological hallmark with respect to the other pathological hallmarks. The "mediation" package in R software[55] was used to perform the mediation analysis with multiple mediators. The estimation algorithms involve fitting varying coefficient linear structural equations models: one model has the outcome of interest (e.g., CDR-SOB) as the dependent variable and the indicator of *APOE* allele, the multiple

mediators, and the confounders as covariates whereas, for each mediator, there is another model having this mediator variable (e.g., Braak NFT stage) as the dependent variable and the indicator *of APOE* allele, the confounders, and all the other mediators as covariates. A Monte Carlo simulation-based method is then used to estimate the average direct and indirect effects[53]. Because the mediation analysis software that is available does not handle ordinal outcome data, which includes CERAD neuritic plaque scores, Braak NFT stages, extent of CAA, CDR-SOB and MMSE, we treated them as continuous measures. This artificially inflated the power of these analyses (Tables 4 and 5), as it assumes a linear relationship among the ordinal categories of the variables; however, it is likely not problematic for the cognitive outcomes, as they have many ordinal categories that may behave piecewise linearly. For this reason, and because mediation analyses were our primary interest, we did not undertake the ordinal regression analysis for CDR-SOB or MMSE. We treated plaques and NFTs as binary mediator variables in the CDR-SOB and MMSE analyses (cutoff values are presented in footnote of Table 4). In addition, the mediation analyses for the *APOE* alleles ε2 and ε4 were conducted separately, that is, subjects with the ε4 allele were excluded from the ε2 allele mediation analysis, and vice versa.

In this study we did not adjust for potential selection bias associated with the decision to undergo autopsy as we found similar results with and without this adjustment, performed with inverse probability weighting, in our prior study on the NACC autopsy cohort[52].

Results

Description of the sample

The flow-chart in Figure 1 depicts the selection procedure and the number of subjects excluded by each exclusion criterion. As of March 2014, the 2005-2014 NACC autopsy cohort consisted of 2987 subjects, of whom 793 subjects met all the eligibility criteria and did not meet any of the exclusion criteria. The distribution of *APOE* genotypes in this final sample was the following: ε2/ε2=1 (0.1%), ε2/ε3=40 (5.0%), ε3/ε3=339 (42.7%), ε3/ε4=320 (40.3%), and ε4/ε4=93 (11.7%). Allelic frequencies in this AD enriched autopsy cohort were distributed as follows: ε2=2.6%, ε3=65.4%, and ε4=31.9%.

Table 1 shows the demographic characteristics of the study subjects by *APOE* genotype. For the purpose of comparison, Supplemental Table 1 describes the subjects with CDR-SOB=0 who were excluded from the study. Sex and education were not significantly different across genotypes, but there were major differences in AD-related clinical phenotypes across the different genotypes. Besides the well-established association between genotype and age of symptom onset and death (ε4<ε3<ε2), these unadjusted analyses also revealed a strong correlation between genotype and antemortem degree of cognitive impairment (ε4>ε3>ε2). The latter differences were striking despite excluding cognitively intact subjects (CDR-SOB=0): the average MMSE score of *APOE*ε2 carriers was 20.5, whereas the average MMSE score of *APOE*ε4 carriers was only 11.9 for one copy and 9.7 for two copies of the allele.

Table 2 depicts the scores of each AD pathological hallmark as recorded within the NACC database (CERAD score of neuritic plaques, Braak NFT stage, and CAA severity) by *APOE* genotype. Hippocampal sclerosis, arteriosclerosis, atherosclerosis, and Lewy bodies are also presented. The subjects with CDR-SOB=0 excluded from the analyses below are described in Supplemental Table 2 for the purpose of comparison. Major differences between genotypes can be observed in the summary data of Table 2: among *APOE*ε2 carriers with impaired cognition at death, just fewer than half (18 out of 41, ∼44%) had sparse or no neuritic plaques whereas this number is almost 10-fold lower for *APOE*ε4 carriers (21 out of 413, ∼5%). Similarly, only ∼27% of *APOE*ε2 carriers (11 out of 41) were Braak V/VI, the most severe NFT stages, whereas almost 90% of *APOE*ε4/4 carriers (81 out of 93) had this widespread distribution of NFTs. Last, moderate or severe CAA was present in only ∼27% of *APOE*ε2 carriers (10 out of 41), as opposed to ∼72% of *APOE*ε4/ε4 carriers (67 out of 93).

Next we examined the complex relationships between *APOE* alleles, AD neuropathology, and cognitive performance prior to death.

Effects of APOE alleles on AD pathological hallmarks

To investigate the independent effects of the *APOE*ε2 and the *APOE*ε4 alleles on the presence and severity of each of these AD pathological hallmarks, we constructed multivariable models including all three pathological lesions and all demographic and clinical variables (age of death, sex, education, and symptom duration), using the *APOE*ε3/ε3 group as reference group (Table 3). While individuals with a primary neuropathological diagnosis of Lewy body disease, hippocampal sclerosis, and cerebrovascular disease were excluded from the study, the “incidental” finding of these lesions was allowed. However, univariate analyses in this group of cognitively impaired (CDR-SOB>0) individuals revealed no correlation of Lewy bodies, hippocampal sclerosis, arteriosclerosis (parenchymal small vessel disease), or atherosclerosis of large arteries with carrying either of the *APOE* alleles. Therefore these neuropathological variables were not included in the multivariable models.

Effect of APOE ε2 and ε4 alleles on neuritic plaques—After controlling for age of death, sex, education, symptom duration, Braak stage of NFTs, and severity of CAA, there was a significant positive association between the presence of the *APOE*ε4 allele and the density of neuritic plaques as assessed with CERAD scores compared to the *APOE*ε3/ε3 genotype, with a higher density in *APOE*ε4 carriers. By contrast, compared to the *APOE*ε3/ε3 group, the presence of the *APOE*ε2 allele was not significantly associated with a lower density of neuritic plaques (although see further analysis and discussion below).

Effect of APOE ε2 and ε4 alleles on cerebral amyloid angiopathy—Similarly, after controlling for age of death, sex, education, symptom duration, Braak stage of NFTs, and CERAD score of neuritic plaques, there was a significant positive association between the presence of the *APOE*ε4 allele and the risk of CAA, with a higher risk of having mild versus none CAA and moderate versus mild CAA, but not severe versus moderate CAA, in *APOE*ε4 carriers compared to the *APOE*ε3/ε3 group. Split analyses by number of *APOE*ε4

alleles revealed a clear dose-dependent association. Carrying one *APOE*ε4 allele was associated with a higher risk of mild but not moderate or severe CAA, whereas carrying two *APOE*ε4 alleles was associated with a higher risk of having moderate and severe but not mild CAA. By contrast, compared to the *APOE*ε3/ε3 genotype, there was no significant association between the presence of the *APOE*ε2 allele and the risk of CAA.

Effect of APOE ε2 and ε4 alleles on neurofibrillary tangles—Neither the presence of the *APOE*ε4 allele nor the number of *APOE*ε4 alleles was significantly associated with a higher risk of having more advanced Braak stages than the *APOE*ε3/ε3 group, when controlling for age of death, sex, education, symptom duration, CERAD score of neuritic plaques, and CAA severity. By contrast, carrying an *APOE*ε2 allele was significantly associated with a lower risk of having a higher Braak stage of NFTs, compared to the *APOE*ε3/ε3 genotype.

Pathobiological pathways linking APOE alleles with AD pathological hallmarks

The above results indicate that, compared to the most common genotype *APOE*ε3/ε3, the *APOE*ε2 allele is independently associated with a lower Braak NFT stage, whereas the *APOE*ε4 allele is independently associated with both a higher density of neuritic plaques and a higher severity of CAA. To investigate possible pathobiological pathways leading from these *APOE* alleles to their pathological correlates, we performed separate mediation analyses with each of the three AD pathological hallmarks as outcome variables, and the other two as mediators (Table 4). All analyses were adjusted by age of death, sex, and education, and *APOE*ε4 analyses were also adjusted by symptom duration, presence of hippocampal sclerosis, and severity of arteriosclerosis (ischemic small vessel disease). The *APOE*ε2 analyses could not adjust for these additional variables due to the small sample size.

These mediation analyses revealed that the protective effect of the *APOE*ε2 allele against NFTs can be explained by two mechanisms: a direct effect of the *APOE*ε2 allele and an indirect pathway mediated through its effect on neuritic plaques, both with similar magnitude. Of note, these analyses also suggested a protective effect of the *APOE*ε2 allele against neuritic plaques resulting from two effects of similar size: a direct effect against neuritic plaques and an indirect consequence of its protective effect on NFTs. Mediation analyses also revealed that the *APOE*ε2 allele has no significant direct effect on CAA severity but does have a significant indirect protective effect mediated through its lowering of both neuritic plaques and NFTs.

The accumulation of neuritic plaques associated with the *APOE*ε4 allele is the result of a direct effect and, to a lesser extent, of an indirect pathway involving its effect on NFTs, but not on CAA. Similarly, the increasing severity of CAA associated with the *APOE*ε4 allele stems mainly from a direct effect, although its effects on neuritic plaques and NFTs also contribute to some extent. Last, the *APOE*ε4 allele has no direct effect on NFTs, but may promote NFTs formation and/or spreading by indirect pathways involving its significant enhancing effects on neuritic plaques and, to a lesser extent, CAA.

Lack of independent effect of APOEε2 and ε4 alleles on cognition

We have recently reported in this same autopsy cohort that the extent of neuritic plaques (as measured by CERAD scores) and NFTs (as measured by Braak stage), severe CAA, severe parenchymal small vessel disease, and the presence of hippocampal sclerosis, all independently predict a higher degree of antemortem cognitive impairment as measured by CDR-SOB, whereas education level is an independent protective factor against cognitive impairment[52]. Herein, we examined whether the *APOE* genotype impacts cognition independently of both demographics and neuropathology. Specifically, we tested the hypothesis that possessing the *APOE*ε2 allele directly or indirectly protects against cognitive deficits, whereas carrying the *APOE*ε4 allele directly or indirectly contributes to cognitive impairment, as captured by CDR-SOB score or MMSE score prior to death. To distinguish between an effect mediated by neuropathological variables (indirect) or an independent (direct) effect of each allele, mediation analyses were carried out controlling for all the demographic/clinical (age of death, sex, education, age of onset, and symptom duration) and neuropathological characteristics. Although the total effect of *APOE*ε2 on CAA was non-significant (Table 4), the effect was not negligible (0.14) and the confidence interval was wide and non-symmetric (-0.53,0.26). For these reasons, in spite of the non-significance, we considered it as a mediator of *APOE*ε2 in the CDR-SOB model. Because the *APOE*ε2 and ε4 alleles are known to have opposite effects on age of onset of cognitive decline —*APOE*ε2 delays onset whereas *APOE*ε4 anticipates it—, we also tested age of onset as a mediator of indirect effects of *APOE* alleles on cognition.

Neither the *APOE*ε2 allele nor the *APOE*ε4 allele had a significant independent (direct) impact on cognition as measured with CDR-SOB and as compared to the more common genotype *APOE*ε3/ε3 (Table 5). Similar results were observed using the MMSE score as the cognitive outcome. However, the *APOE*ε2 allele did exert an indirect protective effect against cognitive impairment that was mediated through its lowering effect on NFTs and, to a lesser extent, through its lowering effect on neuritic plaques. CAA could not be tested as a mediator of *APOE*ε2 allele-induced cognitive effects due to instability of the model. The protective effect on antemortem cognition associated with the *APOE*ε2 allele was not mediated by the delayed age of onset also associated with this allele. Conversely, the *APOE*ε4 allele had an indirect deleterious effect on cognition mediated by all three AD neuropathological lesions. In addition, the effect of *APOE*ε4 on the age of onset of cognitive decline significantly contributed to the severity of cognitive impairment prior to death, even after controlling for symptom duration.

Discussion

The examination of raw data and unadjusted statistical analyses describing this large cognitively impaired autopsy cohort (Tables 1 and 2) suggested that, compared to the most common *APOE*ε3/ε3, carrying the *APOE*ε4 allele may be associated with both a more severe clinical expression (i.e., younger age of onset and poorer cognition prior to death) and a more advanced pathological phenotype (i.e., higher scores for neuritic plaques and CAA) of AD, whereas carrying the *APOE*ε2 allele may imply developing a clinically and pathologically milder form. This would be in agreement with the known opposite effects of

these *APOE* alleles on the risk of developing AD. However, the relationships between genotype and phenotype can be so complex that robust statistical methods and multivariable analyses are necessary to account for confounding and mediator factors. The results of these analyses support the above interpretation and can be summarized as follows: 1) Compared to *APOE*ε3/ε3, *APOE*ε2 is independently associated with both lower Braak stages of NFTs and fewer neuritic plaques, but has no independent effect on CAA severity; 2) compared to *APOE*ε3/ε3, *APOE*ε4 is associated with higher numbers of neuritic plaques and higher severity of CAA, but has no independent effect on NFTs; 3) besides these direct effects of *APOE* ε2 and ε4 alleles on each AD lesion, additional indirect effects mediated through the other two AD pathological hallmarks were identified; 4) neither *APOE*ε2 nor *APOE*ε4 has an independent impact on cognition prior to death, but each indirectly affects cognition through its effects on AD neuropathological changes.

The main novel finding of this study is a significant negative direct effect of the *APOE*ε2 allele on the Braak stage of NFT, suggesting a protective effect of the apolipoprotein E2 against NFT formation and spreading, whereas *APOE*ε4 does not exhibit the opposite effect. Mediation analyses indicated that two effects equally contributed to this negative association between *APOE*ε2 and NFTs: a direct effect of *APOE*ε2 and an indirect mechanism through its plaque-lowering effect. Several proposed pathophysiological mechanisms could underlie these findings. First, apolipoprotein E (apoE) may directly interact with tau protein, the main constituent of NFTs, and apoE2 may interact with tau to a different extent than the other apoE isoforms[56–60]. Second, *APOE*ε2 may indirectly contribute to restrict NFT spreading to cortical areas and determine a lower Braak stage by protecting against the accumulation of soluble Aβ oligomeric species —probably not well captured by the CERAD neuritic plaque score— and plaques[61] and by protecting against synaptotoxicity and neurotoxicity of existing plaques downstream Aβ accumulation[61,62]. By contrast, the association of *APOE*ε4 allele and Braak stage of NFT was not significant after controlling for plaques and CAA, arguing against a significant direct effect of the *APOE*ε4 allele on NFT formation and spreading. Indeed, in agreement with prior work[36,63], our mediation analyses suggested that the positive association between the *APOE*ε4 allele and the Braak NFT stage was largely explained by the *APOE*ε4 allele effect on plaques.

The mediation analys also demonstrate that the augmentation of neuritic plaques and CAA induced by the *APOE*ε4 allele was primarily a direct effect, supporting its role as initiator of amyloid deposition both at the brain parenchyma and at the blood vessel wall in the form of CAA. This result is in agreement with numerous postmortem quantitative and amyloid PET studies that have established that the increased risk of AD induced by *APOE*ε4 is associated with a higher amyloid burden. Recent experimental data indicate that the apoE4 increases the amount of synaptotoxic soluble Aβ oligomeric species[61,64,65] and impairs Aβ clearance[61,66].

While the ordinal regression model in Table 3 detected a protective effect of the *APOE*ε2 allele against neuritic plaques when compared to the *APOE*ε3/ε3, this association was not found to be statistically significant likely due to lack of power, as suggested by the upper limit of the 95% confidence interval for the log odds ratio just above zero. Mediation analyses did yield a statistically significant result, although this statistical significance was

likely contributed by the greater power afforded by the mediation analysis due to its modeling of CERAD neuritic plaque score as continuous, rather than ordinal. In addition, due to the small sample size, the *APOE*ε2 mediation analysis was not able to control for symptom duration, which may be a confounder also contributing to this statistical significance given its significant association with a higher Braak stage ($p<0.0001$) and marginally significant association with a higher CERAD score ($p=0.07$) in the ordinal regression models. In any case, the protective effect of the *APOE*ε2 allele against neuritic plaques was equally explained by a direct effect on plaques and an indirect mechanism, mediated through its NFT-lowering effect. This latter indirect effect appears to imply a synergistic interaction between tangles and plaques that has been postulated based on experimental data[67,68].

Although significant indirect protective effects of the *APOE*ε2 allele on CAA severity were detected by mediation analysis, the total and direct effects were not significant, and the association between *APOE*ε2 allele and CAA severity was not significant in the ordinal regression model. This neutral total effect of the *APOE*ε2 allele on the extent of CAA is consistent with prior work suggesting that the *APOE*ε2 allele is not associated with a higher incidence of vascular amyloid deposition[26,69]. The current NACC autopsy data set did not allow us to confirm the previously described association between the *APOE*ε2 allele and a higher incidence of vasculopathic changes in amyloid-β-laden vessels leading to a higher risk of CAA-related hemorrhages.

Whether the *APOE* genotype independently and directly influences the rate of cognitive decline in AD has been a matter of controversy. Multiple clinical studies have attributed to the *APOE*ε4 allele either an accelerating, a neutral[70–77], or even a slowing effect[78–80] on the rate of cognitive decline once AD has been diagnosed. By contrast, compared to the *APOE*ε3/ε3 genotype, the *APOE*ε2 allele has been associated with a reduced decline[81,82]. However, all these clinical studies lacked autopsy confirmation and, therefore, were not able to study the effect of the *APOE* genotype on cognition in the context of the neuropathological lesions. This is of critical importance because up to 30% of subjects diagnosed with probable AD in US NIA-funded ADCs have other neuropathological diagnoses causing or contributing to their cognitive deficits, or insufficient AD neuropathological changes at autopsy to fulfill current AD neuropathological diagnostic criteria[83,49,48,84]. In this study *APOE*ε2 predicted substantially less severe cognitive impairment at the time of death, whereas *APOE*ε4 was associated with a more severe cognitive impairment. However, these effects were seemingly entirely mediated by the impact of both alleles on AD neuropathological changes. Indeed, we observed no significant direct effect (i.e., independent of pathological lesions) of either of the *APOE* alleles on the CDR-SOB or MMSE score prior to death. We did, however, observe significant indirect effects of both *APOE* alleles on cognition mediated by their opposite effects with respect to the accumulation of AD neuropathological lesions. In addition, part of the detrimental effect of the *APOE*ε4 allele on antemortem cognition was mediated by its effect on the age of onset of cognitive decline. The *APOE*ε4 allele is known to anticipate the onset of cognitive decline in AD, and *APOE*ε4 carriers exhibited a more severe cognitive impairment prior to death the younger they were at clinical onset, even after controlling for symptom duration.

In order to prevent “false” statistically significant results derived from an overrepresentation of the *APOE*ε2 allele and an under representation of the *APOE*ε4 allele among the cognitively intact subjects, we decided to exclude subjects with CDR-SOB=0 and focused our statistical analyses in the continuum of cognitive decline ranging from subjective cognitive complaints and mild cognitive impairment (CDR-SOB=0.5-3.0) to end-stage dementia (CDR-SOB=18). While this conservative design reinforces the validity of the statistically significant results obtained, the power to detect other statistically significant and clinically relevant associations may have been hampered by a smaller sample size, particularly for the *APOE*ε2 allele group (n=41) and for the detection of early direct or indirect effects of *APOE* alleles on cognition.

In summary, the analysis of this large autopsy sample of cognitively impaired elderly subjects selected to examine the clinico-pathological continuum of Alzheimer disease with regression and mediation models led us to the novel finding that the *APOE*ε2 allele is independently associated with a lower Braak NFT stage. This observation implies a protective effect of the *APOE*ε2 allele against spreading of neurofibrillary tangle pathology to the neocortex and warrants future experimental studies modeling the interaction between apolipoprotein E and tau. We also confirmed the association of the *APOE*ε4 allele with a higher density of neuritic plaques and a more severe degree of CAA. These data also emphasize the impact of the *APOE* genotype in the clinical presentation and course of AD, with the major impact of the *APOE*ε4 allele on amyloid variables and a surprisingly strong impact of *APOE*ε2 on the spread of NFTs to the cortex leading to clinically meaningful differences in severity of dementia at death. Thus, *APOE*ε2 appears to lead to a milder form of AD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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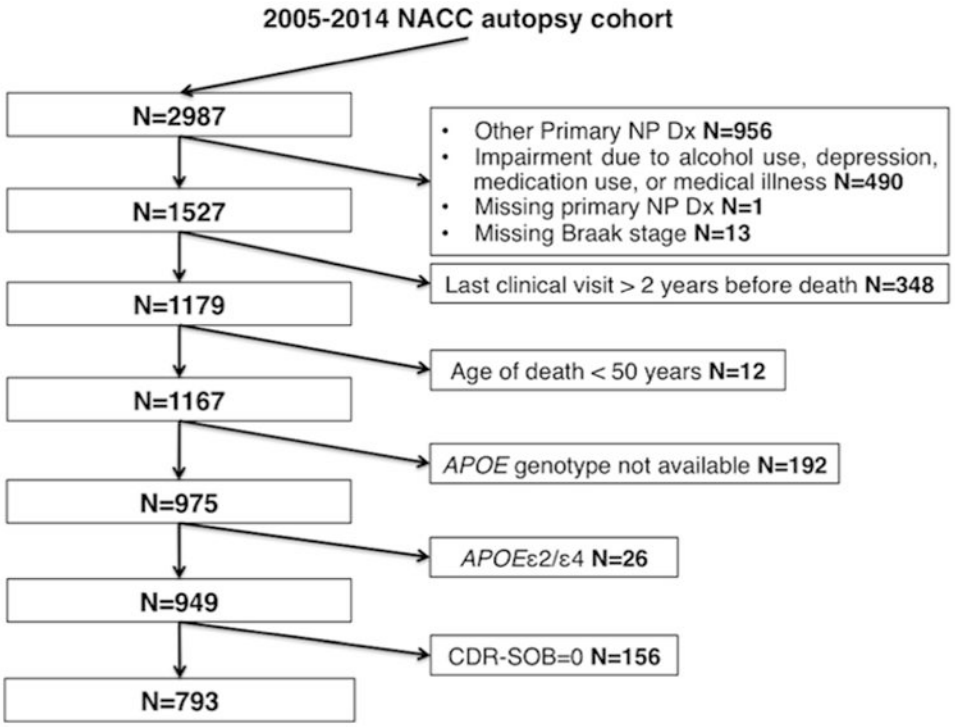

Figure 1.

Flow-chart of the selection process from March 2014 data freeze of the National Alzheimer's Coordinating Center (NACC) autopsy cohort. Other primary NP Dx: other primary neuropathological diagnoses refer to conditions other from Alzheimer disease and includes frontotemporal lobar degeneration, progressive supranuclear palsy, corticobasal degeneration, dementia with Lewy bodies, Parkinson disease, hypoxia, hemorrhage/ hematoma, necrosis, vascular dementia, hippocampal sclerosis, and prion-associated diseases.

Table 1

Demographic and clinical characteristics of the cognitively impaired subjects (CDR-SOB>0) by *APOE* genotype

	ε2/ε2+ε2/ε3	ε3/ε3	ε3/ε4	ε4/ε4	*P* value
Number of subjects, n	41	339	320	93	NA
Sex, n female (%)	21 (51.2)	163 (48.1)	128 (40.0)	40 (43.0)	0.155
Education (y)	14.5±3.5	14.9±3.3	14.9±3.2	15.1±3.2	0.955
Age of death (y)	84.4±12.1	82.9±10.6	81.3±9.1	78.6±8.0	<0.0001[hj]
Age of onset (y)	77.6±14.0	73.4±12.6	71.5±10.5	68.7±8.7	<0.0001[bhj]
Symptom duration (y)	6.8±4.5	9.2±6.3	9.7±6.5	9.7±4.4	0.012 [bc]
CDR-SOB	6.0 (2.0-12.0)	13.0 (6.0-18.0)	16.0 (9.5-18.0)	17.0 (12.0-18.0)	<0.0001[eklm]
MMSE	20.5±9.1	15.3±9.6	11.9±8.8	9.7±8.3	<0.0001[ailmo]

Values represent mean±SD except for CDR-SOB that is expressed as median (interquartile range), and sex that is indicated as number (%) of females. Comparisons were performed with Kruskal-Wallis ANOVA with Dunn's multiple comparison test, except for sex, that was compared using Chi-square with Fisher´s exact test.

[a] $p<0.05$ ε2/ε2+ε2/ε3 vs ε3/ε3;

[b] $p<0.05$ ε2/ε2+ε2/ε3 vs ε3/ε4;

[c] $p<0.05$ ε2/ε2+ε2/ε3 vs ε4/ε4;

[d] $p<0.05$ ε3/ε3 vs ε3/ε4;

[e] $p<0.05$ ε3/ε3 vs ε4/ε4;

[f] $p<0.01$ ε2/ε2+ε2/ε3 vs ε3/ε3;

[g] $p<0.01$ ε2/ε2+ε2/ε3 vs ε3/ε4;

[h] $p<0.01$ ε2/ε2+ε2/ε3 vs ε4/ε4;

[i] $p<0.01$ ε3/ε3 vs ε3/ε4;

[j] $p<0.01$ ε3/ε3 vs ε4/ε4;

[k] $p<0.0001$ ε2/ε2+ε2/ε3 vs ε3/ε3;

[l] $p<0.0001$ ε2/ε2+ε2/ε3 vs ε3/ε4;

[m] $p<0.0001$ ε2/ε2+ε2/ε3 vs ε4/ε4;

[n] p <0.0001 ε3/ε3 vs ε3/ε4;

[o] p<0.0001 ε3/ε3 vs ε4/ε4.

CDR-SOB = clinical dementia rating scale sum of boxes; MMSE = Mini Mental State Examination; NA = not applicable.

Table 2

Pathological characteristics of the cognitively impaired subjects (CDR-SOB>0) by *APOE* genotype

	ε2/ε2+ε2/ε3	ε3/ε3	ε3/ε4	ε4/ε4	*P* value
Number of subjects, n	41	339	320	93	NA
Neuritic plaques, n (%)					<0.0001
None/sparse	18 (43.9)	49 (14.5)	20 (6.3)	1 (1.1)	
Moderate	12 (29.3)	97 (28.6)	47 (14.7)	13 (14.0)	
Frequent	11 (26.8)	193 (56.9)	253 (79.1)	79 (85.0)	
Braak NFT stage, n (%)					<0.0001
0/I/II	13 (31.7)	35 (10.3)	15 (4.7)	0 (0.0)	
III/IV	17 (41.5)	82 (24.2)	56 (17.5)	12 (12.9)	
V	4 (9.8)	84 (24.8)	91 (28.4)	26 (28.0)	
VI	7 (17.1)	138 (40.7)	158 (49.4)	55 (59.1)	
CAA, n (%)					<0.0001
None	20 (48.8)	133 (39.2)	53 (16.6)	12 (12.9)	
Mild	9 (22.0)	101 (29.8)	104 (32.5)	14 (15.1)	
Moderate	6 (14.6)	64 (18.9)	100 (31.3)	32 (34.4)	
Severe	5 (12.2)	35 (10.3)	54 (16.9)	35 (37.6)	
Not assessed/unknown	1 (2.4)	6 (1.8)	9 (2.8)	0 (0.0)	
Arteriosclerosis, n (%)					0.887
None	8 (19.5)	66 (19.5)	46 (14.4)	15 (16.1)	
Mild	11 (26.8)	96 (28.3)	88 (27.5)	26 (28.0)	
Moderate	11 (26.8)	74 (21.8)	76 (23.8)	19 (20.4)	
Severe	3 (7.3)	26 (7.7)	27 (8.4)	10 (10.8)	
Not assessed/unknown	8 (19.5)	77 (22.7)	83 (25.9)	23 (24.7)	
Atherosclerosis, n (%)					0.829
Yes	35 (85.4)	281 (82.9)	261 (81.6)	79 (85.0)	
No	6 (14.6)	58 (17.1)	59 (18.4)	14 (15.0)	
Hippocampal sclerosis, n (%)					0.560
Yes	2 (4.9)	26 (7.7)	30 (9.4)	7 (7.5)	

	ε2/ε2+ε2/ε3	ε3/ε3	ε3/ε4	ε4/ε4	*P* value
No	37 (90.2)	298 (87.9)	286 (89.4)	83 (89.3)	
Not assessed/unknown	2 (4.9)	15 (4.4)	4 (1.3)	3 (3.2)	
Lewy bodies, n (%)					0.121
Yes	9 (22.0)	87 (25.7)	112 (35.0)	29 (31.2)	
No	32 (78.0)	250 (73.8)	208 (65.0)	64 (68.8)	
Not assessed/unknown	0 (0.0)	2 (0.6)	0 (0.0)	0 (0.0)	

Numbers in parenthesis represent column percents, that is, percent of subjects of each *APOE* genotype with a given neuropathologic feature. CAA = cerebral amyloid angiopathy. NA = not applicable. NFT = neurofibrillary tangles. *P* value is for comparison of the distribution of each neuropathological variable among *APOE*ε2 carriers (ε2/ε2+ε2/ε3) and *APOE*ε4 carriers (ε3/ε4+ε4/ε4).

Table 3

Associations between the *APOE* alleles and AD pathological lesions

	Outcome									
	Braak stage of NFT[a]		CERAD score of neuritic plaques[b]		CAA[c]					
					Mild versus none		*Moderate versus mild*		*Severe versusmoderate*	
	Log [OR (95%CI)]	***P* value**	**Log[OR (95%CI)]**	***P* value**	**Log[OR (95%CI)]**	***P* value**	**Log[OR (95%CI)]**	***P* value**	**Log[OR (95%CI)]**	***P* value**
Presence of *APOE*ε2 allele	**-0.25 (-0.49, -0.01)**	**0.04**	-0.19 (-0.48, 0.09)	0.178	0.09 (-0.10, 0.28)	0.33	0.09 (-0.10, 0.28)	0.33	0.09 (-0.10, 0.28)	0.33
Presence of *APOE*ε4 allele	-0.08 (-0.18, 0.02)	0.14	**0.33 (0.17, 0.48)**	**<0.001**	**0.38 (0.19, 0.57)**	**<0.001**	**0.20 (0.01, 0.39)**	**0.038**	0.04 (-0.18, 0.26)	0.71
Presence of 1 *APOE*ε4 allele	-0.08 (-0.18, 0.03)	0.15	**0.31 (0.15, 0.48)**	**<0.001**	**0.44 (0.25, 0.64)**	**<0.001**	0.05 (-0.06, 0.17)	0.36	0.05 (-0.06, 0.17)	0.36
Presence of 2 *APOE*ε4 alleles	-0.08 (-0.24, 0.09)	0.38	**0.39 (0.09, 0.69)**	**<0.001**	0.17 (-0.21, 0.56)	0.38	**0.43 (0.11, 0.75)**	**0.008**	**0.36 (0.08, 0.63)**	**0.010**

Effect estimates represent the logarithm of odd ratios. The logarithm of odd ratios was calculated to facilitate comparisons with the estimates resulting from mediation analysis in table 4.

[a] For Braak stage of NFTs, estimates refer to the following comparisons: VI versus V, V versus III/IV, and III/IV versus 0/I/II.

[b] For CERAD score of neuritic plaques, estimates refer to the following comparisons: frequent versus moderate and moderate versus none/sparse.

[c] For CAA, estimates refer to the comparisons indicated in the table. With each one of the three pathological lesions as dependent variable, the model adjusted for the other two of the three pathological lesions, and all demographic and clinical variables (age of death, sex, education, and symptom duration), using the *APOE*ε3/ε3 group as reference group.

Presence of *APOE*ε2 allele: *APOE*ε2/ε2 + *APOE*ε2/ε3 (but not *APOE*ε2/ε4) versus *APOE*ε3/ε3 (reference); presence of *APOE*ε4 allele: *APOE*ε3/ε4 + *APOE*ε4/ε4 (but not *APOE*ε2/ε4) versus *APOE*ε3/ε3 (reference); CAA = cerebral amyloid angiopathy; NFT = neurofibrillary tangles.

Table 4

Mediation analyses of the possible pathobiological pathways linking *APOE* alleles with AD pathological hallmarks

Outcome	Presence of *APOEε2* allele		Presence of *APOEε4* allele	
Neuritic plaques	**Estimate (95% CI)**	***P* value**	**Estimate (95% CI)**	***P* value**
Total effect	-0.85 (-1.25, -0.46)	<0.001	0.33 (0.18, 0.48)	<0.001
Direct effect	-0.42 (-0.77, -0.09)	0.02	0.23 (0.11, 0.35)	<0.001
Indirect effects:				
through NFTs	-0.42 (-0.60, -0.25)	<0.001	0.10 (0.02, 0.17)	0.008
through CAA	-0.01 (-0.04, 0.03)	0.56	-0.001 (-0.03, 0.02)	0.96
Neurofibrillary tangles	**Estimate (95% CI)**	***P* value**	**Estimate (95% CI)**	***P* value**
Total effect	-1.33 (-1.92, -0.71)	<0.001	0.30 (0.06, 0.54)	0.02
Direct effect	-0.60 (-1.16, -0.06)	0.03	0.06 (-0.12, 0.26)	0.51
Indirect effects:				
through plaques	-0.72 (-1.14, -0.31)	<0.001	0.19 (0.04, 0.35)	0.006
through CAA	-0.01 (-0.09, 0.06)	0.69	0.05 (0.01, 0.10)	0.01
Cerebral amyloid angiopathy	**Estimate (95% CI)**	***P* value**	**Estimate (95% CI)**	***P* value**
Total effect	-0.14 (-0.53, 0.26)	0.41	0.53 (0.36, 0.69)	<0.001
Direct effect	0.19 (-0.18, 0.54)	0.31	0.45 (0.30, 0.61)	<0.001
Indirect effects:				
through plaques	-0.15 (-0.29, -0.03)	0.006	0.04 (0.004, 0.08)	<0.001
through NFTs	-0.19 (-0.33, -0.05)	<0.001	0.04 (0.003, 0.08)	0.01

Effect estimates represent coefficients from linear models. Cutoffs for mediators were selected based on our prior findings of pathological correlates of cognition in NACC autopsy cohort[52], as follows: for neuritic plaques, none/sparse versus moderate/frequent; for Braak NFT stage, 0 to IV versus V/VI, and for CAA, none/mild versus moderate/severe. For the *APOEε2* model, mediators include two of the three AD pathological hallmarks other than the one as dependent variable; additionally, the model is adjusted for age of death, gender, and education as confounders. *APOEε4* analyses are similar to *APOEε2*, but additionally adjusted for symptom duration, presence of hippocampal sclerosis, and severity of arteriosclerosis (ischemic small vessel disease).

Presence of *APOEε2* allele: *APOEε2/ε2* + *APOEε2/ε3* (but not *APOEε2/ε4*) versus *APOEε3/ε3* (reference); presence of *APOEε4* allele: *APOEε3/ε4* + *APOEε4/ε4* (but not *APOEε2/ε4*) versus *APOEε3/ε3* (reference); CAA = cerebral amyloid angiopathy; NFT = neurofibrillary tangles.

Table 5

Impact of *APOE* alleles on cognition (CDR-SOB and MMSE) and mediators of their impact

	CDR-SOB			
	Presence of *APOE ε2* allele		**Presence of *APOEε4* allele**	
	Estimate (95% CI)	***P* value**	**Estimate (95% CI)**	***P* value**
Total effect	-2.89 (-4.77, -0.93)	0.004	0.62 (-0.17, 1.35)	0.10
Direct effect	-0.76 (-2.56, 0.99)	0.42	-0.29 (-0.98, 0.32)	0.36
Indirect effects:				
through neuritic plaques	-0.82 (-1.56, -0.09)	0.004	0.24 (0.08, 0.41)	<0.001
through NFTs	-1.65 (-2.50, -0.79)	<0.001	0.34 (0.12, 0.57)	<0.001
through CAA	NA	NA	0.16 (0.00, 0.33)	0.05
through age of onset	-0.32 (-0.88, 0.25)	0.24	0.19 (0.03, 0.35)	0.01
	MMSE			
	Presence of *APOE ε2* allele		**Presence of *APOEε4* allele**	
	Estimate (95% CI)	***P* value**	**Estimate (95% CI)**	***P* value**
Total effect	3.49 (-0.13, 6.47)	0.07	-3.10 (-4.35, -1.80)	0.002
Direct effect	-0.33 (-3.15, 2.60)	0.83	-0.98 (-2.13, 0.18)	0.13
Indirect effects:				
through neuritic plaques	1.17 (0.11, 2.24)	0.01	-0.33 (-0.59, -0.07)	0.002
through NFTs	2.42 (1.10, 3.74)	<0.001	-0.66 (-1.09, -0.22)	0.004
through CAA	NA	NA	-0.56 (-0.93, -0.19)	<0.001
through age of onset	0.33 (-0.63, 1.30)	0.46	-0.62 (-1.00, -0.24)	<0.001

Effect estimates represent coefficients from linear models. For the *APOEε2* model, mediators include two AD pathological hallmarks (neuritic plaques and NFTs) and age of onset, whereas confounders include gender, education, symptom duration, and CAA. We could not include CAA as a mediator for *APOEε2* due to the small sample size of this group. For the *APOEε4* model, mediators include the three AD pathological hallmarks (neuritic plaques, NFTs, and CAA) and age of onset, whereas confounders include gender, education, and symptom duration.

Presence of *APOEε2* allele: *APOEε2/ε2* + *APOEε2/ε3* (but not *APOEε2/ε4*) versus *APOEε3/ε3* (reference); presence of *APOEε4* allele: *APOEε3/ε4* + *APOEε4/ε4* (but not *APOEε2/ε4*) versus *APOEε3/ε3* (reference); CAA = cerebral amyloid angiopathy; CDR-SOB = clinical dementia rating scale sum of boxes; MMSE = Mini Mental State Examination; NFT = neurofibrillary tangles.