Published in final edited form as:

Alzheimer Dis Assoc Disord. 2009 ; 23(2): 152–157.

The Effect of APOE- ϵ 4 on Dementia is Mediated by Alzheimer Neuropathology

James A. Mortimer, Ph.D.,

Department of Epidemiology and Biostatistics, College of Public Health, University of South Florida, Tampa, FL

David A. Snowdon, Ph.D., and

Department of Neurology and Sanders Brown Center on Aging, University of Kentucky, Lexington, KY

William R. Markesbery, M.D.

Departments of Neurology and Pathology and Sanders Brown Center on Aging, University of Kentucky, Lexington, KY

Abstract

The degree to which the association of ϵ 4 with dementia is mediated by AD lesions in comparison with vascular lesions is controversial. The present study was undertaken to determine the roles of Alzheimer (AD) and vascular pathology in mediating the effect of APOE- ϵ 4 alleles on dementia. Clinicopathologic correlations were studied in 267 Catholic sisters participating in the Nun Study. The extent to which AD and vascular pathologies mediated the effect of APOE- ϵ 4 on dementia was investigated using multiple logistic regression. Adjusted for age at death and education, possession of one or more ϵ 4 alleles was an important risk factor for dementia (OR=2.98, 95% CI: 1.62-5.48). This association was lost (OR=1.38, 95% CI: 0.68-2.80) when an index of the severity of AD-related neuropathology was added to the model, but changed little when measures of the severity of vascular pathology were added. The findings suggest that the effect of ϵ 4 on dementia is mediated by the severity of AD pathology. While infarcts and atherosclerosis contribute to the occurrence of dementia, this contribution appears unrelated to APOE genotype.

Keywords

Apolipoprotein E; Alzheimer's disease; AD neuropathology; vascular lesions; clinicopathologic correlation

Introduction

At autopsy, demented patients show considerable overlap in pathological lesions in the brain, with Alzheimer's disease (AD) and vascular lesions commonly found.^{1,2} The heterogeneity of pathology raises the question of how these different lesions might mediate the effects of the ϵ 4 allele of apolipoprotein E (APOE), an important risk factor for dementia³ and AD.³⁻⁸

In the Religious Orders Study, when the effect of AD pathology was controlled in regression analyses, the association of the ϵ 4 allele with clinical AD was reduced by 50%.⁹ This finding suggests that much of the effect of this allele on AD was through an association with the

classical neuropathologic lesions of this illness. The Religious Orders Study compared participants with probable AD to controls and excluded persons with other types of dementia, in whom $\epsilon 4$ may also have influenced non-AD pathology. In the present study, all cases of dementia were included.

The degree to which the association of $\epsilon 4$ with dementia is mediated by vascular lesions in comparison with AD lesions is unknown. The possibility that vascular lesions may play a role in mediating the effects of the $\epsilon 4$ allele is raised by studies showing associations between APOE- $\epsilon 4$ and increased risk of vascular dementia.¹⁰⁻¹³ as well as by a recent study in which APOE- $\epsilon 4$ was shown to more than double the odds of cortical and subcortical infarction.¹⁴

We used data from 267 participants in the Nun Study who came to autopsy to examine the roles of AD and vascular pathology in mediating the effect of APOE- $\epsilon 4$ alleles. Participants were included regardless of the presence or cause of dementia. Our analyses closely parallel those from the previous Religious Orders Study publication⁹ in determining the effect of adding measures of pathology on the association between the $\epsilon 4$ allele and dementia in regression models.

Methods

Participants

Participants in the Nun Study^{15,16} are members of the School Sisters of Notre Dame congregation and live in communities in the Midwestern, Eastern, and Southern United States. In 1991 and 1992, 678 Catholic sisters, aged 75 to 102 were enrolled who agreed to annual evaluation and brain donation after death. Participants included individuals who were initially demented and those free of dementia and other cognitive impairments. Cognitive and physical function was assessed annually. All participants or their guardians gave informed consent to their participation in this study. Ninety-two percent of those enrolled continued participation and 96% of those who died were autopsied. The present study is based on the first 412 participants who were autopsied. Data on both APOE genotype and ratings of amyloid angiopathy were available for 267 of these participants, who constituted the sample for the present analyses.

Assessment During Life

Cognitive function was evaluated annually with the CERAD neuropsychological battery.¹⁷ This battery assesses memory, concentration, language, visuospatial ability, and orientation to time and place. Performance-based testing was used to evaluate basic and instrumental activities of daily living.^{18,19} Participants were considered to be demented when all of the following conditions were met: 1) impairment in memory (defined by a score <4 on the Delayed Word Recall Test); 2) impairment in one or more other areas of cognition (defined by scores of <11 on Verbal Fluency, <13 on Boston Naming or <8 on Constructional Praxis); 3) impairment in basic or instrumental activities of daily living (defined by inability to use a telephone, handle money, or dress oneself); and 4) evidence of a decline in ability from a previous level attributable to cognitive impairment. Cut scores selected to define cognitive impairment on tests were based on scores less than the fifth percentile for normal controls in CERAD.²⁰

APOE Genotyping

APOE genotyping was performed using genetic material from buccal cells collected from living participants or from frozen or paraffin-embedded brain specimens using laboratory methods described previously.²¹

Neuropathologic Evaluation

Gross and microscopic evaluations of the brains of participants who died during the study follow-up period were performed by a board-certified clinical neuropathologist (WRM) who was blinded to diagnosis, cognitive test scores and functional assessments. Diffuse and neuritic plaques (NP) and neurofibrillary tangles (NFT) were counted in the five most severely affected fields of the middle frontal gyrus (Brodmann area 9), inferior parietal lobule (areas 39/40), middle temporal gyrus (area 21), and the CA1 and subiculum of the hippocampus after staining with the modified Bielschowsky method. The Gallyas stain, which is better for detecting neuropil threads and argyrophilic grains and slightly better for detecting NFT in medial temporal lobe structures, was used to count the neurofibrillary pathology in CA1 and subiculum of the hippocampus. All sections were cut at 8 micron thickness.

To meet the study's neuropathologic criteria for AD, participants were required to have: 1) abundant senile plaques in the frontal, temporal, or parietal lobes, i.e., 16 or more senile plaques per mm²; 2) neuritic plaques in at least one lobe; and 3) NFT in at least one lobe and abundant NFT in the entorhinal cortex, hippocampus and amygdala. Participants meeting the study's neuropathologic criteria all satisfied CERAD criteria for neuropathological AD and had a high or intermediate likelihood of neuropathological AD according to the National Institute on Aging/Reagan Institute guidelines.

Cortical and subcortical infarcts were identified at the gross examination of the intact brain and of 1.5 cm thick coronal sections of the cerebral hemispheres, brainstem, and cerebellum. They were categorized as large (≥ 1.5 cm.) or lacunar (< 1.5 cm.). The degree of atherosclerosis in the circle of Willis was rated in four grades from absent to severe. Because there is no universally accepted staging of atherosclerosis in the circle of Willis, the scale used reflects the subjective impression of the study neuropathologist (WRM) regarding the overall average of involvement of the number of the affected vessels at the base of the brain and the degree of atherosclerotic depositions in these vessels. In the present study, 13% of participants had absent to mild atherosclerosis (plaques present in 0% to less than 25% of the vessel walls of the circle of Willis), 33% moderate (25% to 50% inclusive) and 54% severe ($> 50\%$).

Chronic microinfarcts were identified from slides of the caudate nucleus, putamen, globus pallidus, thalamus, internal capsule, frontal lobe (area 9), temporal lobe, parietal lobe, occipital lobe (areas 18 and 19), and hippocampus.

The severity of amyloid angiopathy was rated from 1 (absent) to 4 (severe). The number of blood vessels containing beta amyloid by immunostaining was counted in six regions, including the leptomeninges and the cortex of the frontal, parietal, temporal, and occipital lobes and hippocampus. Six or more blood vessels containing amyloid in greater than 50% of the wall in three or more brain regions and leptomeninges was considered severe. Moderate was considered when two or more regions and leptomeninges had six or more affected vessels, and mild when one region showed six or more affected vessels. If the severity was less than this, amyloid angiopathy was considered to be absent. Amyloid angiopathy determined in this manner was absent in 24%, mild in 43%, moderate in 5% and severe in 28%.

Statistical Methods

Differences between participants with and without one or more $\epsilon 4$ alleles for individual descriptive variables were assessed with Wilcoxon rank-sum and chi square tests. An exploratory principal components analysis partialing out the effects of age at death and years of education was used to identify factors from the seven pathological measures obtained in the study. Preliminary analyses showed that none of the correlations between measures exceeded 0.5, permitting the inclusion of ordinal as well as dichotomous variables²². Logistic regression

was used to examine the associations between the presence of one or more APOE- ϵ 4 alleles and dementia, controlling for age at death and years of education. The effect of indices representing the severity of Alzheimer pathology and vascular pathology was then investigated by adding these indices to the models with APOE- ϵ 4, age at death, and education, and comparing the strength of associations between APOE- ϵ 4 and dementia when these indices were present and absent. Additional models were run in which individual pathological measures were added to APOE- ϵ 4, age at death and education. Finally, associations of the seven pathological measures with each other were assessed by Chi square, rank biserial correlation (r_{tb}) or Spearman correlation (r_s), depending on the types of variables.

Results

Table 1 compares characteristics of the 200 participants without an ϵ 4 allele and the 67 participants with one or more ϵ 4 alleles. Those with one or more ϵ 4 alleles had higher mean counts of neocortical NFT, higher mean counts of neocortical neuritic plaques, and higher ratings of the severity of amyloid angiopathy. In addition, they were more likely to be demented at death, and to fulfill study neuropathologic criteria for AD. Other measures, including the four non-amyloid vascular measures, were not significantly different.

Principal components analysis of the seven pathological measures revealed three factors with eigenvalues greater than one. The first factor had loadings of 0.62 (mean number of neocortical NFT), 0.59 (mean number of neocortical NP), 0.51 (rating of amyloid angiopathy severity), 0.07 (lacunar infarcts), 0.07 (large infarcts), -0.01 (presence of microinfarcts) and -0.06 (rating of severity of atherosclerosis in the circle of Willis). The second factor had loadings of 0.61 (large infarcts), 0.55 (lacunar infarcts), 0.45 (rating of severity of atherosclerosis in the circle of Willis), 0.33 (presence of microinfarcts), 0.09 (rating of amyloid angiopathy severity), -0.06 (mean number of neocortical NFT) and -0.10 (mean number of neocortical NP). The third factor had only one variable loading greater than .40: 0.76 (presence of microinfarcts). Two indices were created by summing standardized values of neocortical NFT, neocortical NP and amyloid angiopathy to yield an *AD pathology index*; and by summing standardized values of atherosclerosis and large and lacunar infarcts to yield a *vascular pathology index*. Because the third factor consisted of a single variable, the presence of microinfarcts was added independently to the models.

Table 2 summarizes multiple logistic regression models with dementia as the outcome. APOE- ϵ 4 was strongly associated with dementia (OR=2.98, 95% CI:1.62-5.48) adjusted for age at death and years of education (Model 1). When the AD pathology index was added to the model, the association of APOE- ϵ 4 with dementia was reduced and lost its statistical significance (OR=1.38, 95%CI: 0.68-2.80) (Model 2). By contrast when the vascular pathology index was added, the association of ϵ 4 with dementia changed only slightly (Model 3). The addition of microinfarcts (Model 4) led to a small increase in the odds ratio for APOE- ϵ 4. Finally, in the full model (Model 5), both the AD and vascular pathology indices were independently associated with dementia.

To assess the role of individual pathologies, a series of logistic regression analyses were performed in which the APOE- ϵ 4 association with dementia was evaluated with addition of each pathological measure alone (Table 3). Addition of the mean count of neocortical NFT eliminated the significant association of APOE- ϵ 4 with dementia; none of the other six measures eliminated this significant association. However, inclusion of the mean count of neocortical neuritic plaques reduced the magnitude of the APOE- ϵ 4 odds ratio from 2.98 to 2.22.

Significant associations were found between the presence of large and lacunar infarcts ($\chi^2=29.3$, $p<0.0001$), large infarcts and ratings of atherosclerosis in the circle of Willis ($r_{rb}=.30$, $p<0.01$), and lacunar infarcts and atherosclerosis in the circle of Willis ($r_{rb}=.21$, $p<0.05$). However, there were no significant associations between ratings of amyloid angiopathy and the occurrence of large ($r_{rb}=.13$, NS) or lacunar ($r_{rb}=.09$, NS) infarcts, between ratings of amyloid angiopathy and atherosclerosis ($r_s=-.06$, NS), between ratings of amyloid angiopathy and microinfarcts ($r_s=.14$, NS), between microinfarcts and either lacunar ($\chi^2=1.16$, NS) or large ($\chi^2=2.41$, NS) infarcts, or between microinfarcts and atherosclerosis ($r_{rb}=.13$, NS). The rating of amyloid angiopathy was significantly correlated with both the mean NFT count in the neocortex ($r_s=.36$, $p<0.0001$) and the mean NP neocortical count ($r_s=.33$, $p<0.0001$).

Discussion

Consistent with previous research,³ presence of one or more APOE- $\epsilon 4$ alleles was a strong predictor of dementia. The addition of the index of severity of Alzheimer pathology to the model reduced the strength of this association from 2.98 to 1.38, suggesting that the effect of APOE- $\epsilon 4$ on dementia is largely accounted for by more severe AD neuropathology. Addition of an index of cerebrovascular disease, including the degree of atherosclerosis in the circle of Willis and the presence of large and lacunar infarcts, as well as addition of chronic microinfarcts had little effect on the association between the $\epsilon 4$ allele and dementia.

Among the three AD-related pathologies, only the mean count of neocortical neurofibrillary tangles when added to a model predicting dementia from APOE- $\epsilon 4$, age at death and education substantially reduced the association between $\epsilon 4$ and dementia. This finding suggests that APOE- $\epsilon 4$ influences the occurrence of dementia primarily through its association with more severe NFT expression, and is consistent with the results of many clinicopathologic studies including the Nun Study²³, which found a stronger association of dementia with the frequency of neurofibrillary tangles than with the frequency of neuritic plaques.

Our findings for AD neuropathology are in general agreement with those of the Religious Orders Study (ROS)⁹, which used a similar approach to analyze data. In that study, the likelihood of clinical AD was strongly associated with the $\epsilon 4$ allele in logistic regression models and the association was reduced approximately 50% by addition of a summary measure of global AD pathology reflecting diffuse plaque, neuritic plaque and neurofibrillary tangle densities. However, in contrast to the findings of that study, we did not see a large reduction in the association of $\epsilon 4$ with dementia by addition of a measure of mean neocortical neuritic plaque count. Although the measures of neuritic plaque density were similar between the two studies, there was a large difference in age at death. Participants in the present study were on average more than 5 years older at the time of their death than those in the ROS. While the ROS investigators reported similar associations with dementia of normalized measures of neurofibrillary tangles (OR=3.59, 95CI: 1.69-7.60) and neuritic plaques (OR=3.90; 95%CI: 1.99-7.61); normalized measures of neurofibrillary tangles and neuritic plaques in the present study demonstrated much stronger associations of neurofibrillary tangles with dementia (OR=5.17; 95%CI: 2.76-9.71) as compared to neuritic plaques (OR=1.89; 95%CI: 1.42-2.50). It is possible that the difference in findings may be accounted for by increased progression of the AD pathologic process,²⁴ with neurofibrillary tangles playing a more important role in those who survived to older ages.

A recent publication based on data from the Religious Orders Study²⁵ examined the extent to which a dichotomous variable (presence or absence of chronic cerebral infarction) mediated the effect of APOE- $\epsilon 4$ on continuous measures of cognitive function. In agreement with the results of the present study, the authors did not find a significant mediation effect of chronic infarction on the association between APOE- $\epsilon 4$ and global cognition.

Autopsy-based studies have shown significant correlations between the $\epsilon 4$ allele and the density of neuritic plaques,²⁶⁻²⁸ NFT,^{27,29-31} and amyloid deposition.²⁹⁻³⁴ However, some studies have not found an association with amyloid plaques³⁵⁻³⁷ or NFT.^{26,32,35,37,38} With few exceptions, those studies not finding an association were restricted to individuals with clinically diagnosed dementia, while those showing an association included substantial numbers of nondemented elderly. The lack of an association seen in some studies may represent a ceiling effect achieved with increasing disease severity.³⁹ The Catholic sisters that are the focus of this report cover a wide spectrum of both cognitive performance and neuropathology, which may have facilitated finding stronger associations between $\epsilon 4$ and AD pathology.

Studies of clinically diagnosed vascular dementia patients have been equivocal with regard to associations with APOE- $\epsilon 4$. Some have shown elevated $\epsilon 4$ frequencies,¹⁰⁻¹³ whereas others demonstrated normal frequencies of this allele.⁴⁰⁻⁴⁴ Similarly, in clinical studies the association of the $\epsilon 4$ allele and stroke has been mixed, with some studies reporting a positive association between APOE- $\epsilon 4$ and stroke,⁴⁵⁻⁴⁷ while others have failed to confirm this association.⁴⁸⁻⁵³ Studies in which strokes were identified by autopsy are relatively few. Two studies^{54,55} found that the frequency of cerebrovascular lesions was not increased in APOE- $\epsilon 4$, while another group¹⁴ reported that APOE- $\epsilon 4$ significantly increased the odds of both cortical and subcortical infarctions. In the present study, we did not find a statistically-significant association of APOE- $\epsilon 4$ with the presence of large infarcts or lacunar infarcts, the degree of atherosclerosis in the circle of Willis or the presence of chronic microinfarcts. However, the degree of atherosclerosis in the circle of Willis was significantly associated with the presence of both large and lacunar infarcts, and both types of infarcts contributed to the expression of dementia as we have shown previously for the same cohort.¹⁶

The association between cardiovascular disease, atherosclerosis and AD has been controversial.⁵⁶⁻⁵⁹ Our results suggest that cerebrovascular and AD lesions do not share APOE- $\epsilon 4$ as a common risk factor. Rather they support the hypothesis that vascular lesions increase the clinical expression of an AD process that is accelerated by possession of one or more $\epsilon 4$ alleles. An important exception is amyloid angiopathy, which was strongly related to AD neuropathology, but had no association with the severity of atherosclerosis or the occurrence of infarcts.

In summary, the present results suggest the effect of $\epsilon 4$ on dementia is mediated almost entirely by the severity of AD pathology. Although infarcts contribute significantly to the occurrence of dementia, this contribution appears unrelated to APOE genotype.

This study has several strengths. These include the ability to compare dementia status before death to detailed measures of AD and vascular pathology at autopsy in a large population genotyped for APOE. Little attrition occurred during the study and the autopsy rate was high, limiting these sources of bias. Because approximately half of the sisters died without becoming demented and a wide spectrum of both AD and vascular pathology was present at autopsy, there was significant power to assess associations between the presence of $\epsilon 4$ alleles, dementia and vascular and AD lesions. Blinding of the neuropathologist to all clinical data eliminated bias due to expectations based on premorbid status.

Limitations include the restriction of AD neuropathologic measures to mean counts of neocortical neurofibrillary tangles and neuritic plaques and ratings of severity of amyloid angiopathy. Because we did not know the pathological burden from sisters when they were alive, the sequence of events from APOE genotype to clinical presentation must be inferred from cross-sectional regression analyses. Finally, the study was conducted entirely in women with a high degree of education and lifestyles that limited exposure to tobacco and alcohol as well as other confounders. Because higher education reduces expression of dementia,⁶⁰ a less

well-educated sample might be more likely to become demented with less severe neuropathology, resulting in somewhat different associations between APOE- ϵ 4, AD neuropathology and dementia. Our findings, while largely consistent with those of another study of well-educated Catholic nuns, priests and brothers⁹, need to be replicated in a sample of lay persons with a greater range of educational attainment.

Acknowledgments

This study would not have been possible without the spirited support of the members, leaders, and health care providers of the School Sisters of Notre Dame religious congregation. This study was funded by grants P50-AG025711 (Dr. Mortimer), R01-AG09862 (Dr. Snowdon) and 1P30-AG28383 (Dr. Markesbery) from the National Institute on Aging.

References

1. Xuereb JH, Brayne C, DuFouil C, et al. Neuropathological findings in the very old. Results from the first 101 brains of a population-based longitudinal study of dementing disorders. *Ann NY Acad Sci* 2000;903:490–496. [PubMed: 10818543]
2. White L, Small BJ, Petrovitch H, et al. Recent clinical-pathologic research on the causes of dementia in late life: Update from the Honolulu-Asia Aging study. *J Geriatr Psychiatry Neurol* 2005;18:224–227. [PubMed: 16306244]
3. Henderson AS, Eastel S, Jorm AF, et al. Apolipoprotein E allele epsilon 4, dementia, and cognitive decline in a population sample. *Lancet* 1995;346:1387–1390. [PubMed: 7475820]
4. Myers RH, Schaefer EJ, Wilson PW, et al. Apolipoprotein E ϵ 4 association with dementia in a population-based study: The Framingham Study. *Neurology* 1996;46:673–677. [PubMed: 8618665]
5. Evans D, Beckett L, Field R, et al. Apolipoprotein E ϵ 4 and incidence of Alzheimer's disease in a community population of older persons. *JAMA* 1997;277:822–824. [PubMed: 9052713]
6. Farrar LA, Cupples LA, Haines JL, et al. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer's disease: a meta analysis. *JAMA* 1997;278:1349–1356. [PubMed: 9343467]
7. Breitner JCS, Jarvik GP, Plassman BL, Saunders AM, Welsh KA. Risk of Alzheimer disease with the ϵ 4 allele for apolipoprotein E in a population-based study of men aged 62–73 years. *Alzheimer Dis Assoc Disord* 1998;12:40–44. [PubMed: 9539409]
8. Tang MX, Stern Y, Marder K, et al. The APOE- ϵ 4 allele and the risk of Alzheimer disease among African Americans, whites and Hispanics. *JAMA* 1998;279:751–755. [PubMed: 9508150]
9. Bennett DA, Wilson RS, Schneider JA, et al. Apolipoprotein E ϵ 4 allele, AD pathology, and the clinical expression of Alzheimer's disease. *Neurology* 2003;60:246–252. [PubMed: 12552039]
10. Shamano H, Ishibashi S, Murase T, et al. Plasma apolipoproteins in patients with multi-infarct dementias. *Atherosclerosis* 1989;79:257–260. [PubMed: 2597234]
11. Frisoni GB, Calabresi L, Geroldi C, et al. Apolipoprotein E epsilon 4 allele in Alzheimer's disease and vascular dementia. *Dementia* 1995;5:240–242. [PubMed: 7951679]
12. Engelborghs S, Dermaut B, Goeman J, et al. Prospective Belgian study of neurodegenerative and vascular dementia: APOE genotype effects. *J Neurol Neurosurg Psychiatry* 2003;74:1148–1151. [PubMed: 12876259]
13. Davidson Y, Gibbons L, Purandare N, et al. Apolipoprotein E ϵ 4 allele frequency in vascular dementia. *Dement Geriatr Cogn Disord* 2006;22:15–19. [PubMed: 16645276]
14. Schneider JA, Bienias JL, Wilson RS, et al. The apolipoprotein E ϵ 4 allele increases the odds of chronic cerebral infarction detected at autopsy in older persons. *Stroke* 2005;36:954–959. [PubMed: 15774818]
15. Snowdon DA, Kemper SJ, Mortimer JA, et al. Linguistic ability in early life and cognitive function and Alzheimer's disease in late life: findings from the Nun Study. *JAMA* 1996;275:528–532. [PubMed: 8606473]
16. Snowdon DA, Greiner LH, Mortimer JA, et al. Brain infarction and the clinical expression of Alzheimer disease. *JAMA* 1997;277:813–817. [PubMed: 9052711]

17. Morris JC, Heyman A, Mohs RC, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part I. Clinical and neuropsychological assessment of Alzheimer's disease. *Neurology* 1989;39:1159–1165. [PubMed: 2771064]
18. Kuriensky J, Gurland B. The performance test of activities of daily living. *Int J Aging Hum Develop* 1976;7:343–352.
19. Potvin AR, Tourtellotte WW, Dailey JS, et al. Simulated activities of daily living examination. *Arch Phys Med Rehab* 1972;53:476–489.
20. Welsh KA, Butters N, Mohs RC, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part V. A normative study of the neuropsychological battery. *Neurology* 1994;44:609–614. [PubMed: 8164812]
21. Saunders AM, Hulette C, Welsh-Bohmer KA, et al. Specificity, sensitivity, and predictive value of apolipoprotein E genotyping for sporadic Alzheimer's disease. *Lancet* 1996;348:90–93. [PubMed: 8676723]
22. Wildt, AR.; Mueller, CW.; Kim, JO. Introduction to Factor Analysis: What It Is and How To Do It. Thousand Oaks, CA: Sage Publications, Inc; 1978.
23. Riley KP, Snowdon DA, Markesbery WR. Alzheimer's neurofibrillary pathology and the spectrum of cognition function: Findings from the Nun Study. *Ann Neurol* 2002;51:567–577. [PubMed: 12112102]
24. Bennett DA, Schneider JA, Wilson RS, Biennas JL, Berry-Kravis E, Arnold SE. Amyloid mediates the association of apolipoprotein E $\epsilon 4$ allele to cognitive function in older people. *J Neurol Neurosurg Psychiatry* 2005;76:1194–1199. [PubMed: 16107349]
25. Li Y, Schneider JA, Bennett DA. Estimation of the mediation effect with a binary mediator. *Statist Med* 2007;26:2298–3414.
26. Olichney JM, Hansen LA, Galasko D, et al. The apolipoprotein E $\epsilon 4$ allele is associated with increased neuritic plaques and cerebral amyloid angiopathy in Alzheimer's disease and Lewy body variant. *Neurology* 1996;47:190–196. [PubMed: 8710076]
27. Warzok RW, Kessler C, Apel G, et al. Apolipoprotein E4 promotes incipient Alzheimer pathology in the elderly. *Alzheimer Dis Assoc Disord* 1998;12:33–39. [PubMed: 9539408]
28. Yip AG, McKee AC, Green RC, et al. APOE, vascular pathology, and the AD brain. *Neurology* 2005;65:259–265. [PubMed: 16043796]
29. Ohm TG, Kirca M, Bohl J, et al. Apolipoprotein E polymorphism influences not only cerebral senile plaque load but also Alzheimer-type neurofibrillary tangle formation. *Neuroscience* 1995;66:583–587. [PubMed: 7644022]
30. Polvikoski T, Sulkava R, Haltia M, et al. Apolipoprotein E, dementia, and cortical deposition of beta-amyloid protein. *NEJM* 1995;333:1242–1247. [PubMed: 7566000]
31. Ohm TG, Scharnagl H, Marz W, Bohl F. Apolipoprotein E isoforms and the development of low and high Braak stages of Alzheimer's disease-related lesions. *Acta Neuropathol* 1999;98:273–280. [PubMed: 10483785]
32. Gomez-Isla T, West HL, Rebeck GW, et al. Clinical and pathological correlates of apolipoprotein E $\epsilon 4$ in Alzheimer's disease. *Ann Neurol* 1996;39:62–70. [PubMed: 8572669]
33. Morishima-Kawashima M, Oshima N, Ogata H, et al. Effect of apolipoprotein E allele $\epsilon 4$ on the initial phase of amyloid beta-protein accumulation in the human brain. *Am J Pathol* 2000;157:2093–2099. [PubMed: 11106581]
34. Walker LC, Pahnke J, Madauss M, et al. Apolipoprotein E4 promotes the early deposition of A β 42 and then A β 40 in the elderly. *Acta Neuropathol* 2000;100:36–42. [PubMed: 10912918]
35. Heinonen O, Lehtovirta M, Soininen H, et al. Alzheimer pathology of patients carrying apolipoprotein E $\epsilon 4$ allele. *Neurobiol Aging* 1995;16:505–513. [PubMed: 8544899]
36. Morris CM, Massey HM, Benjamin R, et al. Molecular biology of APO E alleles in Alzheimer's and non-Alzheimer's dementias. *J Neur Transm Suppl* 1996;47:205–218.
37. Landen M, Thirsell A, Wallin A, Blennow K. The apolipoprotein E allele epsilon 4 does not correlate with the number of senile plaques or neurofibrillary tangles in patients with Alzheimer's disease. *J Neurol Neurosurg Psychiatry* 1996;61:352–356. [PubMed: 8890772]

38. Mukaetova-Ladinska EB, Harrington CR, Roth M, Wischik CM. Presence of the apolipoprotein E ϵ 4 allele is not associated with neurofibrillary pathology or biochemical changes to tau protein. *Dement Geriatr Cogn Disord* 1997;8:288–295. [PubMed: 9298630]
39. Hansen LA, Galasko D, Samuel W, et al. Apolipoprotein E epsilon-4 is associated with increased neurofibrillary pathology in the Lewy body variant of Alzheimer's disease. *Neurosci Lett* 1994;182:63–65. [PubMed: 7891889]
40. Betard C, Robitaille Y, Gee M, et al. Apo E allele frequencies in Alzheimer's disease, Lewy body dementia, Alzheimer's disease with cerebrovascular disease and vascular dementia. *Neuroreport* 1994;5:1893–1896. [PubMed: 7841371]
41. Palumbo B, Parnetti L, Nocentini G, et al. Apolipoprotein-E genotype in normal aging, age-associated memory impairment, Alzheimer's disease and vascular dementia patients. *Neurosci Lett* 1997;231:59–61. [PubMed: 9280168]
42. Wehr H, Bednarska-Makaruk M, Lojkowska W, et al. Differences in risk factors for dementia with neurodegenerative traits and for vascular dementia. *M Dement Geriatr Cogn Disord* 2006;21:1–7.
43. Orsitto G, Seripa D, Panza F, et al. Apolipoprotein E genotypes in hospitalized elderly patients with vascular dementia. *Dement Geriatr Cogn Disord* 2007;23:327–333. [PubMed: 17374951]
44. Kim KW, Youn JC, Han MK, et al. Lack of association between apolipoprotein E polymorphism and vascular dementia in Koreans. *J Geriatr Psychiatry Neurol* 2008;21:12–17.
45. McCarron MO, Delong D, Alberts MJ. APOE genotype as a risk factor for ischemic cerebrovascular disease. *Neurology* 1999;53:1308–1311. [PubMed: 10522889]
46. Kim JS, Han SR, Chung SW, et al. The apolipoprotein E ϵ 4 haplotype is an important predictor for recurrence in ischemic cerebrovascular disease. *J Neurol Sci* 2003;206:31–37. [PubMed: 12480082]
47. Jin ZQ, Fan YS, Ding J, et al. Association of apolipoprotein E4 polymorphism with cerebral infarction in Chinese Han population. *Acta Pharmacol Sin* 2004;25:352–356. [PubMed: 15000890]
48. Couderc R, Mahieux F, Bailleul S. Apolipoprotein E allele frequency, ischemic cerebrovascular disease and Alzheimer's disease. *Stroke* 1994;24:1416–1417.
49. Kuusisto J, Mykkanen L, Kervinen K, Kesaniemi YA, Laakso M. Apolipoprotein E4 phenotype is not an important risk factor for coronary heart disease or stroke in elderly subjects. *Arterioscler Thromb Vasc Biol* 1995;15:1280–1286. [PubMed: 7670939]
50. Basun H, Corder EH, Guo Z, et al. Apolipoprotein E polymorphism and stroke in a population sample aged 75 years or more. *Stroke* 1996;27:1310–1315. [PubMed: 8711793]
51. Kuller LH, Shemanski L, Manolio T, et al. Relationship between apoE, MRI findings, and cognitive function in the Cardiovascular Health Study. *Stroke* 1998;29:388–398. [PubMed: 9472879]
52. Frikke-Schmidt R, Nordestgaard BG, Thudium D, Moes Gronholdt ML, Tybjaerg-Hansen A. APOE genotype predicts AD and other dementia but not ischemic cerebrovascular disease. *Neurology* 2001;56:194–200. [PubMed: 11160955]
53. MacLeod MJ, De Lange RP, Breen G, et al. Lack of association between apolipoprotein E genotype and ischaemic stroke in a Scottish population. *Eur J Clin Invest* 2001;31:570–573. [PubMed: 11454010]
54. Olichney JM, Hansen LA, Hofstetter CR, et al. Association between severe cerebral amyloid angiopathy and cerebrovascular lesions in Alzheimer disease is not a spurious one attributable to apolipoprotein E4. *Arch Neurol* 2000;57:869–874. [PubMed: 10867785]
55. Chui HC, Zarow C, Mark WJ, et al. Cognitive impact of subcortical vascular and Alzheimer's disease pathology. *Ann Neurol* 2006;60:677–687. [PubMed: 17192928]
56. Sparks DL, Scheff SW, Liu H, et al. Increased density of senile plaques (SP), but not neurofibrillary tangles (NFT), in non-demented individuals with the apolipoprotein E4 allele: comparison to confirmed Alzheimer's disease patients. *J Neurol Sci* 1996;138:97–104. [PubMed: 8791246]
57. Irina A, Seppo H, Arto M, Riekkinen P, Soininen H. β -amyloid load is not influenced by the severity of cardiovascular disease in aged and demented patients. *Stroke* 1999;30:613–618. [PubMed: 10066860]
58. Roher AE, Esch C, Kokjohn TA, et al. Circle of Willis atherosclerosis is a risk factor for sporadic Alzheimer's disease. *Arterioscler Thromb Vasc Biol* 2003;23:2055–2062. [PubMed: 14512367]
59. Beeri MS, Rapp M, Silverman JM, et al. Coronary artery disease is associated with Alzheimer disease neuropathology in APOE4 carriers. *Neurology* 2006;66:1399–1404. [PubMed: 16682673]

60. Mortimer JA, Snowdon DA, Markesbery WR. Head circumference, education and risk of dementia: findings from the Nun Study. *J Clin Exp Neuropsychol* 2003;25:671–679. [PubMed: 12815504]

Table 1
Characteristics of Participants by APOE Genotype

Characteristic	Participants without $\epsilon 4$ Alleles ($n=200$)	Participants with One or More $\epsilon 4$ Alleles ($n=67$)
Education, mean (SD), years	15.7 (3.1)	16.2 (2.7)
Age at death, mean (SD), years	90.8 (5.4)	90.0 (4.8)
Percent demented at death	45.0	67.2 *
Number of neocortical neurofibrillary tangles, mean (SD)	4.2 (10.1)	14.3 (15.3) **
Number of neocortical neuritic plaques, mean (SD)	3.3 (3.4)	4.9 (3.0) **
Percent who fulfilled study criteria for neuropathological AD	51.8	92.3 **
Amyloid angiopathy severity rating, mean (SD)	2.08 (1.01)	3.24 (1.02) **
Rating of atherosclerosis in circle of Willis, mean (SD)	0.46 (.26)	0.45 (0.27)
Percent with one of more large infarcts	17.5	19.4
Percent with one or more lacunar infarcts	33.5	32.8
Percent with chronic microinfarcts	23.4	27.3

Statistical tests: Wilcoxon rank-sum tests were used to compare groups for age at death, number of neocortical neurofibrillary tangles, number of neocortical neuritic plaques, amyloid angiopathy severity rating, and rating of atherosclerosis in the circle of Willis; remaining variables were assessed with the chi square test.

*
 $p < 0.01$

**
 $p < 0.0001$

Table 2
Multiple Logistic Regression Models for Odds of Dementia

Variable	Model 1		Model 2		Model 3		Model 4		Model 5	
	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
ε4 allele	2.98 (1.62-5.48)	<0.001	1.38 (0.68-2.80)	0.37	2.85 (1.54-5.27)	<0.001	3.17 (1.71-5.88)	<0.001	1.29 (0.63-2.68)	0.49
Age at death	1.09 (1.04-1.15)	<0.001	1.09 (1.03-1.15)	<0.01	1.10 (1.04-1.16)	<0.001	1.10 (1.04-1.16)	<0.001	1.08 (1.02-1.15)	<0.01
Years of education	0.90 (0.82-0.99)	<0.05	0.90 (0.82-0.99)	<0.05	0.92 (0.84-1.00)	0.07	0.90 (0.82-0.99)	<0.05	0.92 (0.83-1.01)	0.08
Alzheimer index			1.40 (1.21-1.62)	<0.0001					1.43 (1.23-1.67)	<0.0001
Vascular index					1.15 (1.02-1.30)	<0.05			1.17 (1.02-1.35)	<0.05
Microinfarcts							0.92 (0.70-1.20)	0.54	0.89 (0.66-1.19)	0.42

Abbreviations: OR, odds ratio; CI, confidence interval

Table 3
Multiple Logistic Regression Analyses* for Odds of Dementia with Addition of Individual Pathological Measures to a Model with APOE-ε4

Pathological measure added	Variables	OR (95% CI)	p
None	ε4 allele	2.98 (1.62-5.48)	<0.001
Mean count of neocortical neurofibrillary tangles	ε4 allele	1.10 (0.52-2.34)	0.80
	pathology	1.14 (1.08-1.21)	<0.0001
Mean count of neocortical neuritic plaques	ε4 allele	2.22 (1.18-4.19)	<0.05
	pathology	1.17 (1.08-1.27)	<0.0005
Rating of amyloid angiopathy	ε4 allele	2.90 (1.45-5.76)	<0.01
	pathology	1.05 (0.80-1.36)	0.73
Presence of large cortical infarct(s)	ε4 allele	2.99 (1.62-5.53)	<0.001
	pathology	2.46 (1.23-4.96)	<0.05
Presence of lacunar infarct(s)	ε4 allele	3.04 (1.65-5.63)	<0.001
	pathology	2.11 (1.21-3.65)	<0.01
Degree of atherosclerosis in circle of Willis	ε4 allele	2.82 (1.53-5.21)	<0.001
	pathology	0.89 (0.33-2.38)	0.81
Presence of microinfarcts	ε4 allele	3.17 (1.71-5.88)	<0.001
	pathology	0.78 (0.35-1.73)	0.54

Abbreviations: OR, odds ratio; CI, confidence interval

* All models adjusted for age at death and years of education.