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APOE Predicts Aβ but not Tau Alzheimer's Pathology in Cognitively Normal Aging

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Abstract

Objective—To examine interactions of Apolipoprotein E (*APOE*) genotype with age and with *in vivo* measures of preclinical Alzheimer's disease (AD) in cognitively normal aging.

Methods—Two hundred and 41 cognitively normal individuals, age 45 to 88 years, had cerebral amyloid imaging studies with Pittsburgh Compound-B (PIB). Of the 241 individuals, 168 (70%) also had cerebrospinal fluid (CSF) assays of amyloid-beta$_{42}$ (Aβ_{42}), tau, and phosphorylated tau (ptau$_{181}$). All individuals were genotyped for *APOE*.

Results—The frequency of individuals with elevated mean cortical binding potential (MCBP) for PIB rose in an age-dependent manner from 0% at ages 45-49 years to 30.3% at 80-88 years. Reduced levels of CSF Aβ_{42} appear to begin earlier (18.2% of those age 45-49 years) and increase with age in higher frequencies (50% at age 80-88 years) than elevations of MCBP. There is a gene dose effect for the *APOE4* genotype, with greater MCBP increases and greater reductions in CSF Aβ_{42} with increased numbers of *APOE4* alleles. Individuals with an *APOE2* have no increase in MCBP with age and have higher CSF Aβ_{42} levels than individuals without an *APOE2* allele. There is no *APOE4* or *APOE2* effect on CSF tau or ptau$_{181}$.

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Interpretation—Increasing cerebral Aβ deposition with age is the pathobiological phenotype of *APOE4*. The biomarker sequence that detects Aβ deposition may first be lowered CSF $A\beta_{42}$, followed by elevated MCBP for PIB. A substantial proportion of cognitively normal individuals have presumptive preclinical AD.

Keywords

preclinical Alzheimer's disease; Alzheimer's biomarkers; Aβ; amyloid imaging (PIB); *APOE*

The concept of preclinical Alzheimer's disease (AD) postulates that AD lesions accumulate in the brain for years prior to the appearance of cognitive deficits or symptoms of dementia.1 This concept developed from observations that densities of senile plaques (SPs) and neurofibrillary tangles (NFTs), sufficient to meet histopathologic criteria for AD, frequently are present in brains of individuals whose cognition at death was normal 2-9 or stable.10,11 Preclinical AD assumes that AD neuropathology in cognitively normal individuals results in progressive neuronal deterioration and eventually will culminate in the clinical syndrome of dementia of the Alzheimer type (DAT), although the time to DAT may be influenced by variables such as an individual's degree of cognitive and brain "reserve".12-14 To date, however, it is not known whether DAT is inevitable in preclinical AD. It remains possible that certain individuals with presumptive preclinical AD may never develop DAT, no matter how long they live.

Imaging and molecular biomarkers for AD now identify *in vivo* correlates of neuropathological AD15-20 and can be used as surrogate markers of preclinical AD. In particular, elevations of cerebrospinal fluid (CSF) concentrations of tau or phosphorylated tau (ptau) appear to reflect NFTs in the brain and reduced levels of CSF amyloid-beta ($A\beta_{42}$) appear to reflect cerebral Aβ deposition in the form of plaques.21,22 With positron emission tomography (PET), the [^{11}C] benzothiazole radiotracer, Pittsburgh Compound-B (PIB), is retained in greater amounts in individuals with DAT as compared with cognitively normal persons in brain areas known to have high amounts of fibrillar Aβ plaques in AD. {Klunk, 2004 3704 /id} In postmortem studies, PIB identifies fibrillar Aβ in SPs and in cerebral amyloid angiopathy {Lockhart, 2007 5054 /id;Ikonomovic, 2008 5013 /id} and in human frontal cortical biopsy tissue PIB uptake corresponds to parenchymal Aβ aggregration.25 The correspondence of CSF $A\beta_{42}$ levels with PIB binding values in the same individuals suggests that they each are measuring aspects of cerebral Aβ burden.26,27

These biomarkers for AD may identify nondemented individuals at risk for developing DAT. In persons with mild cognitive impairment (MCI),28 progression to a clinical diagnosis of DAT is predicted by CSF $A\beta_{42}$ and tau levels and PIB findings.29-31 Limited studies in cognitively normal older adults show that reduced levels of CSF $A\beta_{42}$, often combined with elevated levels of tau, predict cognitive decline and dementia,27,32,33 but the value of biomarkers in characterizing preclinical AD antecedent to any measurable cognitive deficit (including MCI) is less studied. Moreover, the relationships of these biomarkers in preclinical AD to established risk factors for AD are unknown.

Increasing age and genetic background are the strongest known risk factors for AD.34 The ε4 allele of Apolipoprotein E (*APOE*), the major genetic susceptibility factor for late-onset AD, confers dramatically increased risk in a gene dose-dependent manner for the development of DAT with an earlier age of onset.35-38 Other isoforms of *APOE* are considered to be neutral (*APOE3*) or protective (*APOE2*) for AD risk.39,40 The increased risk of *APOE4* for AD may be mediated by impaired regulation of cerebral Aβ metabolism.41-43 There is an isoform-dependent propensity (E4>E3>E2) for Aβ to be deposited as cerebral amyloid plaques in experimental animals,44,45 and in humans *APOE4* carriers have

increased cerebral amyloid deposition in comparison with noncarriers.46-48 The role of *APOE4* in promoting clinical disease appears to be directly related to its effect on AD pathology because its association with clinically diagnosed DAT is nonsignificant after controlling for the densities of SPs and NFTs in autopsied individuals.49 Consistent with this premise, an *APOE* genotype effect on Aβ plaque load recently has been demonstrated in amyloid imaging studies in individuals with moderate DAT50 and in nondemented individuals.51,52 However, because of small sample sizes these studies did not address the influence of age on the *APOE4* effect, nor did they examine the role of *APOE2* on Aβ deposition or utilize CSF biomarkers to determine whether *APOE* influences tau or Aβ metabolism.

To more thoroughly examine the interactions of *APOE* with indicators of AD pathology, we examined the relationship of *APOE* genotype with age in 241 cognitively normal individuals, age 45-88 years, with mean cortical binding potential for PIB and, in 168 of the 241 individuals, with CSF levels of $A\beta_{42}$, tau, and ptau. We also wished to determine whether the main effect of APOE is on Aβ or tau. We hypothesized that an effect of age and *APOE* genotype, the known risk factors for AD, on PIB and CSF biomarkers provides biological evidence for the relevance of these biomarkers in characterizing preclinical AD.

METHODS

Participants

Participants were community-living volunteers without cognitive impairment who enrolled in longitudinal studies of memory and aging at Washington University's Alzheimer's Disease Research Center. These studies were inaugurated in 1979 in individuals 60 y or older to evaluate the natural history of mild DAT in comparison with nondemented aging; details about the recruitment and assessment methods have been published.53 Individuals with clinically meaningful disorders (e.g., disabling cerebral infarcts; renal failure requiring dialysis) that could interfere with longitudinal follow-up are excluded. A complementary study, the Adult Children Study (ACS), began in 2005 by enrolling community-living individuals without cognitive impairment, age 45-74 y, into two groups, one in which the biological parent of the participant developed DAT prior to age 80 y, the second in which neither biological parent of the participant had DAT.54 All participants are assessed in a uniform manner with identical instruments and procedures with two exceptions: 1) the ADRC neuropsychological battery is modified for ACS participants to allow for their younger age; and 2) ACS participants are evaluated every three years until age 65 y, when they are evaluated annually (identical to ADRC participants).

Inclusion criteria for this study were: 1) completing PET PIB imaging between April, 2004 and October, 2008; 2) normal cognition at the clinical and cognitive assessment closest in time to the PET PIB scan; and 3) age 45 years or older at that clinical and cognitive assessment. Individuals from families with a known deterministic mutation for AD were excluded.

Evaluation

All procedures were approved by Washington University's Human Research Protection Office. Written informed consent was obtained from all participants and their collateral sources (informants).

At entry and at each follow-up, experienced clinicians conduct semi-structured interviews with the participant and separately with their informant to determine possible decline in the participant's cognitive abilities to function in everyday activities.53,55 Included in the protocol are demographic information, a health history, a depression inventory, an aphasia

battery, and medication history. The Mini Mental State Examination (MMSE)56 also is obtained. Following a neurological evaluation, the clinician determines whether dementia is present or absent based on the principle of intraindividual decline in the performance of accustomed activities because of cognitive loss.55 The absence of dementia corresponds to a Clinical Dementia Rating (CDR) of 0; very mild dementia is indicated by CDR 0.5, and mild, moderate, and severe dementia by CDR 1, 2, and 3.57 Diagnosis of dementia etiology (e.g., DAT) is made in accordance with standard criteria and methods.58 The CDR determination and dementia diagnosis are made without reference to the participant's performance on the neuropsychological test battery.

Within a few weeks of the clinical assessment, a 1.5-hour neuropsychological test battery55 is administered; psychometricians are not informed of the results of the clinical evaluation or of any previous psychometric assessments. The independence of the clinical and the psychometric evaluations allow data obtained with each method to be compared without the confounding that occurs when psychometric performance is used both to classify individuals and to measure outcomes. The clinical assessment procedures permit the detection of minimal cognitive decline, even when the deficits are too mild to meet criteria for MCI,55 and results in the designation of CDR 0.5. The CDR 0 group thus is free of even minimal cognitive impairment.59

CSF collection and analysis

The CSF (20-30 mL) was collected free from any blood contamination in polypropylene tubes at 8:00 AM after overnight fasting as previously described.26 Samples were gently inverted to avoid gradient effects, briefly centrifuged at low speed to pellet any cellular elements, and aliquoted (500 uL) into polypropylene tubes prior to freezing at -84° C. The samples were analyzed for total tau, $ptau_{181}$, and $A\beta_{42}$ by commercial enzyme-linked immunosorbant assay (ELISA) (Innotest, Innogenetics, Ghent, Belgium); CSF Aβ40 was assayed by ELISA as previously described.60 For all biomarker measures, samples were continuously kept on ice and assays were performed on sample aliquots after a single thaw following initial freezing.

Imaging

Detailed information on the PET PIB imaging and analysis procedures have been reported. 61 In brief, brain PET imaging was conducted using a Siemens 961 HR ECAT PET scanner (CTI, Knoxville, KY) or a Siemens 962 HR+ ECAT PET scanner in a darkened, quiet room. A thermoplastic mask was placed to minimize head motion and participants kept their eyes closed during the scan. Radiochemical synthesis of [^{11}C]PIB was carried out according to published literature.62 After a transmission scan to measure attenuation, approximately 12 mCi of [^{11}C]PIB was administered intravenously simultaneous with initiation of a 60-minute dynamic PET scan in three dimensional mode (septa retracted; 24 × 5 seconds frames; 9 × 20 seconds frames; 10 × 1 minute frames). The measured attenuation factors, scatter correction and a ramp filter were employed to reconstruct the dynamic PET images. In addition to PET imaging all participants underwent anatomic magnetic resonance imaging (MRI) using MPRAGE T1-weighted volume (1mm × 1mm × 1.25 mm) acquisitions.

Image Processing

PIB image analysis was performed for specific regions-of-interest (ROIs) as detailed previously.61 This was achieved by first registering each participant's structural MRI to a standard atlas target63 that minimizes bias due to atrophy.64 Detailed information on the boundaries of the ROIs used is available.61 After correction of head motion during the PET scan and alignment of the MRI to the PET PIB scan, the ROIs then were applied to unblurred images of the PET dynamic data, yielding high-resolution regional time-activity

curves. Time-activity curves were analyzed for PIB specific binding by the Logan graphical analysis using the cerebellum ROI data as a reference tissue input function with the linear regression step applied to the data from 30 to 60 minutes.65 The cerebellum was chosen as the reference region because there is little specific binding of PIB in postmortem samples of cerebellar cortex even among those with AD at autopsy.62 The Logan analysis yields a tracer distribution volume ratio (DVratio) which was then converted to an estimate of the binding potential (BP) for each ROI: BP = DVratio − 1.61 The BP expresses the regional PIB binding values in a manner directly proportional to the number of binding sites. The BP values from the prefrontal cortex, gyrus rectus, lateral temporal, and precuneus ROIs were averaged in each subject to calculate a mean cortical binding potential (MCBP) as these regions have been shown to have high PIB uptake among participants with AD.61

Genotyping

DNA was extracted from peripheral blood samples using standard procedures. *APOE* genotyping was performed as previously described.40

Statistical analysis

Age at the time of clinical assessment was used for all analyses involving age. Differences in demographic characteristics between participants with and without CSF data were tested using t-tests for independent samples for continuous variables and using chi-square tests for categorical variables. Scatterplots were used to illustrate associations between each biomarker and age in years as they relate to *APOE4* and *APOE2* genotype. Treating MCBP and CSF $A\beta_{42}$, tau, and $ptau_{181}$ as continuous scales, Analysis of Covariance (ANCOVA) was used to model these biomarkers as a function of age and of number of *APOE4* alleles (0,1, or 2), *APOE2* status (positive vs. negative), sex, race (white vs. non-white), and education in years. Rather than number of *APOE2* alleles, *APOE2* status was used because only 3 individuals had two *APOE2* allele. This method of analysis includes all *APOE* genotypes and allows examination of *APOE4* dose effect while adjusting for all other variables in the model, including the *APOE2* effect, allows examination of the *APOE2* effect while adjusting for *APOE4* dose effects and the other variables in the model, and disentangles the effects of *APOE4* and *APOE2*.

Interactive effects on biomarkers among these factors were examined. Different error variances were fitted to the regression model depending on age to test and, if present, account for increasing variance of the biomarkers as a function of age. Satterthwaite's approximation was used to estimate the denominator degrees of freedom in *F* or *t* tests. These analyses were implemented using PROC MIXED/SAS.66

RESULTS

From April, 2004 through October, 2008, 266 CDR 0 participants had at least one clinical assessment. Of these, 241 were eligible for and completed PET PIB imaging, all within 2 years of the assessment confirming their CDR 0 status. One-hundred sixty-eight of the 241 participants had CSF assays for $A\beta_{42}$, tau, and $ptau_{181}$, also all within 2 years of confirmation of CDR 0 status. The mean interval (standard deviation) between the clinical assessment and PET PIB scan was 0.52 y (0.42 y) and for CSF collection was 0.43 y (0.39 y). The mean interval between PET PIB scan and CSF collection was 0.56 y (0.52 y). Data from many of the 241 participants have appeared in previous reports from our ADRC. 20·61·67

Demographic information is shown in Table 1. The participants ranged in age from 45.2 y to 88.6 y at time of clinical assessment. Participants with only PIB data were older than

participants who had both PIB and CSF data (mean age 71.0 y vs. 64.9 y, p<.0001), but there were no significant differences between participants with and without CSF data with regard to sex (p=.2691), minority race (p=.3371), presence of *APOE4* (p=.5276) or *APOE2* (p=.1840), or mean MMSE scores (p=.1849).

We previously found that participants with DAT typically show MCBP values for PIB at or above 0.18,61 and have CSF $A\beta_{42}$ levels below 500 pg/mL, CSF tau levels above 500 pg/mL, and CSF $ptau_{181}$ levels above 80 pg/mL20,27; our threshold of CSF $A\beta_{42}$ <500 pg/mL is very similar to the optimum cutoff level for antemortem CSF $A\beta_{42}$ of 515 pg/mL as indexed to neuritic plaque burden at autopsy.{Tapiola T, 2009 5230 /id} [In our experience, neither CSF levels of $A\beta_{40}$ nor plasma levels of $A\beta_{40}$ or $A\beta_{42}$ discriminate DAT from healthy aging27 and these markers are not further discussed here.] In the entire sample of 241, 18.3% had MCBP values at or above 0.18. Of the 168 nondemented participants with both PIB and CSF data, the percentage with biomarker values similar to those typically found among participants with DAT were 15.5% for PIB, 28.0% for $A\beta_{42}$, 6.6% for tau, and 4.2% for $ptau_{181}$.

Unadjusted data

For the entire sample, the unadjusted relationships between MCBP for PIB with age as a function of the presence or absence of at least one *APOE4* allele are shown in Figure 1A and as a function of the presence or absence of at least one *APOE2* allele in Figure 1B. For the 168 participants with both PIB and CSF data, the unadjusted associations between CSF $A\beta_{42}$, CSF tau, and CSF $ptau_{181}$ with age as a function of *APOE4* genotype are shown in Figure 2 and as a function of *APOE2* genotype in Figure 3. MCBP increases with age at a faster rate for participants with at least one *APOE4* allele compared with those without an *APOE4* allele (Figure 1A). Individuals with at least one *APOE2* allele have no increase in MCBP with age (Figure 1B). Participants with at least one *APOE4* allele have lower CSF $A\beta_{42}$ values with age than those without *APOE4* (Figure 2A) and those with at least one *APOE2* allele have higher CSF $A\beta_{42}$ values with age (Figure 3A). There is no effect of *APOE4* or *APOE2* on CSF tau or $ptau_{181}$ (Figures 2 B-C and 3 B-C). Figure 4 shows the data presented above when dichotomized into PIB-positive (MCBP > 0.18) and PIB-negative individuals (n=241) and into CSF $A\beta_{42}$-positive (<500 pg/mL) and CSF $A\beta_{42}$-negative individuals (n=168).

For the entire sample, there is an age-related increase in the frequency of abnormal values for MCBP (>0.18) and CSF $A\beta_{42}$ (<500 pg/mL). The frequency of individuals with elevated MCBP for PIB was 0% at age 45-49 years, 5.7% at age 50-59 years, 19.0% at 60-69 years, 25.8% at age 70-79 years, and 30.3% at age 80-89 years. The frequency of individuals with reduced levels of CSF $A\beta_{42}$ is 18.2% at age 45-49 years (only 11 individuals contributed data in this age range), 14.0% at age 50-59 years, 27.1% at age 60-69 years, 34.2% at age 70-79 years, and 50.0% at age 80-89 years (only 14 individuals contributed data in this age range).

Adjusted analyses

As suggested by the scatterplot of MCBP versus age in Figure 1, the variation of MCBP is higher among individuals at least 55 years old when compared to those younger than 55 (χ^2 (1)=68.35, p-value<0.0001). MCBP values increased with greater numbers of *APOE4* alleles; adjusted mean (standard error, SE) MCBP values were 0.033 (0.013), 0.198 (0.021), and 0.417 (0.055) for 0, 1, or 2 *APOE4* alleles (F(2,182)=41.05; p<0.0001). A median split was used to divide the MCBP values into high versus low groups. For individuals with an *APOE4* allele versus those without an *APOE4* allele, the odds for the likelihood of a high MCBP group was 5.04 (95% CI: 2.53-10.2). There is a differential association between

MCBP and age as a function of *APOE4* dosage (F(2,171)=28.07, p<0.0001). For participants with 2 *APOE4* alleles, the MCBP increases by age at an estimated mean of 0.020/year (SE=0.003), whereas the estimated increase is slower for those with 1 *APOE4* allele at 0.013/year (SE=0.001), and the slowest increase in MCBP with age is demonstrated for individuals lacking an *APOE4* allele at 0.003/year (SE=0.001). None of the other factors, including education, sex, and *APOE2* status, are significantly associated with MCBP or affect the detected association between MCBP and *APOE4* and age among nondemented individuals.

APOE4 is associated with lower CSF $A\beta_{42}$ levels, even after adjusting for age and *APOE2* (F(2,162)=11.74; p<0.0001). There is a differential association between CSF $A\beta_{42}$ levels and age in individuals as a function of *APOE2* status (F(1,162)=8.3, p=0.0045) such that CSF $A\beta_{42}$ levels increase nonsignificantly with age in the presence of an *APOE2* allele (4.56 pg/mL per year; SE=4.59) and decrease significantly in the absence of an *APOE2* allele (-7.38 pg/mL per year, SE=1.93). For individuals without an *APOE2* allele, the adjusted mean CSF $A\beta_{42}$ levels are reduced with *APOE4* in dose-dependent manner; the adjusted mean CSF $A\beta_{42}$ level with 0 copies of *APOE4* is 671 pg/mL (SE=24.3) with 1 copy of APOE4 is 588 pg/mL (SE=33.1), and with 2 copies of *APOE4* is 327pg/mL (SE=73.1). None of the other factors, including education and sex, are significantly associated with CSF $A\beta_{42}$ or affect the associations between $A\beta_{42}$ and *APOE2/APOE4* and age.

The variation in both CSF tau and $ptau_{181}$ levels is higher for individuals age 55 y or more compared with those age <55 y (for CSF tau, $\chi^2(1)=9.01$, p=.0027; for CSF $ptau_{181}$, $\chi^2(1)=8.65$, p=0.0033). Age is associated with increasing levels of CSF tau (F(1,130)=39.62, p<.0001) and CSF $ptau_{181}$ (F(1,128)=21.64, p<0.001. None of the other main effects (*APOE4, APOE2*, sex) were significant for CSF tau and CSF $ptau_{181}$.

DISCUSSION

Age and *APOE* genotype interact to increase the frequency of cerebral Aβ deposition in cognitively normal older adults. As measured by reductions in CSF $A\beta_{42}$ levels and by increased MCBP for PIB, we found that cerebral deposition of Aβ begins in middle age and increases in frequency such that 34.2% of individuals age 70-79 years and 50.0% of individuals age 80-89 years in this study had lowered CSF $A\beta_{42}$ levels and 25.8% of individuals age 70-79 years and 30.3% of individuals age 80-89 years had elevated MCBPs. These percentages are comparable to the age-related frequency of neuropathological AD on postmortem examination of cognitively normal older adults9 and correspond to the prevalence of clinically manifest DAT in these age groups,68 as might be expected if cerebral Aβ deposition in asymptomatic older adults denotes preclinical AD that eventually will be expressed as DAT.

We confirm that *APOE4* has a powerful dose-dependent effect on cerebral Aβ deposition with age. (It is clear that age effects also extend to factors other than *APOE4*, as individuals lacking this allele also had age-related elevations in MCBP for PIB and lowered CSF $A\beta_{42}$ levels, although at much lower frequencies than *APOE4* carriers.) The *APOE4* effect persists for the older age groups (>80 y), an unexpected finding given that *APOE4* carriers generally are reported to have an earlier onset of DAT. Possibly *APOE4* has previously unrecognized effects for the expression of DAT in older age groups; alternatively, these older individuals may never become symptomatic with DAT in spite of their *APOE4*-associated increases in cerebral Aβ deposition, although this seems at odds with the comparable frequencies of Aβ deposition with the prevalence of DAT at these ages.

Prior studies of *APOE4* in cognitively normal aging have reported associations with poorer cognitive performance,69-71 reduced cerebral metabolic rates for glucose,72 and smaller regional73,74 and whole brain75 volumes. Recent PET imaging studies with molecular tracers for fibrillar Aβ also report increased cerebral binding of the tracer in normal older adults who carry at least one *APOE4* allele.51,52 Ours is the first study, however, to demonstrate an *APOE4* effect as a function of age on cerebral Aβ and to observe the effect with both molecular Aβ imaging and direct measures of CSF $A\beta_{42}$ concentrations. The concordance of the findings with both measures of Aβ but not with measures of tau or $ptau_{181}$ indicates that cerebral Aβ deposition is the pathobiological phenotype of the *APOE*4 genotype.

To our knowledge, we also provide the first demonstration in cognitively normal older adults of a protective effect of *APOE2* against Aβ deposition as measured both by PIB and CSF $A\beta_{42}$ levels. Because *APOE2* protects against development of DAT, this finding supports a pathogenic role for cerebral Aβ deposition in AD, at least in its preclinical stages. In contrast, CSF levels of tau and $ptau_{181}$ increase as a function of age in cognitively normal older adults but are not affected by *APOE* genotype. The mechanisms by which *APOE4* exerts its dramatically increased risk for AD thus appear to involve Aβ but not tau metabolism, consistent with observations that brain atrophy and longitudinal cognitive decline in nondemented aging correlate with elevated MCBP for PIB{Storandt, 2009 5231 / id} and with abnormalities in CSF $A\beta_{42}$ but not CSF tau or ptau.20 Hence, Aβ is central to the initial detectable pathological changes in preclinical AD, with changes in tau likely occurring later.5

The initial detection of perturbed cerebral Aβ metabolism may be reflected by reduced CSF $A\beta_{42}$ levels, followed by elevated MCBP values for PIB. The data in Figure 4 suggest that CSF $A\beta_{42}$ reductions are shifted to an earlier age and persist in greater frequencies with age than are elevations in MCBP for PIB, but interpretative caution is indicated by the cross-sectional nature of the data. Lowered concentrations of CSF $A\beta_{42}$ perhaps reflect initial Aβ deposition in the brain in the form of diffuse SPs. These initial Aβ deposits, which may be downstream of other Aβ toxic species, such as dimers and oligomers,76 may be largely nonfibrillar and hence unable to bind PIB in concentrations sufficient for detection by PET. Thus, these data suggest that the biomarker sequence for detection of preclinical AD may be an initial reduction of CSF $A\beta_{42}$ levels, followed by elevated MCBP for PIB after the Aβ deposits become fibrillar, but longitudinal studies are needed for a definitive determination of the biomarker sequence for AD.

This study has several strengths. To our knowledge, this is the largest sample (n=241) of older individuals (demented, cognitively normal, or combined) for which PET PIB findings are reported. The Aβ imaging data were combined with CSF levels of $A\beta_{42}$, tau, and $ptau_{181}$ in 70% of the sample. The sample size permitted the first examination of *APOE2* effects on markers of preclinical AD. The wide age span (from 45 to 88 years) allowed examination of the relationships of *APOE* and the AD biomarkers with age. Finally, the 241 participants were carefully characterized to avoid contamination with individuals experiencing even minimal cognitive deficits.

The study also has limitations. We used a convenience sample of individuals willing to be followed longitudinally and intensively (e.g., PET PIB; CSF collection), possibly reducing the generalizability of the findings to the larger population. Interpretative caution is recommended for results involving some cells with small sample sizes, such as the number of individuals (n=34) carrying an *APOE2* allele (the size of our *APOE2* subsample alone, however, is comparable to the size of the total samples used in recent reports of *APOE4* effects on amyloid imaging).50-52 Finally, the concept of preclinical AD must remain

speculative until there is enough evidence that cognitively normal older adults with reduced CSF $A\beta_{42}$, elevated CSF tau, elevated MCBP for PIB, or other indicators for preclinical AD have disproportionately greater risk for developing DAT than those without biomarker abnormalities.

Alzheimer's disease is a complex disorder and its pathogenesis almost certainly cannot be explained simply by abnormal metabolism of Aβ. However, we find powerful evidence that cerebral Aβ deposition with age is the pathobiological phenotype for *APOE4*, the strongest genetic risk factor for late-onset AD, and that the protective effect of *APOE2* against developing AD is mediated by its effects against cerebral Aβ deposition. We also find evidence that Aβ abnormalities, but not tau abnormalities, initiate the pathological cascade of preclinical AD, consistent with our previous findings that reduced levels of CSF $A\beta_{42}$ are associated with low whole brain volumes in nondemented individuals but not those with DAT, whereas elevated CSF tau levels are not associated with low whole brain volumes in nondemented persons but are for those with DAT.{Fagan, 2009 5015 /id}. Finally, reduced levels of CSF $A\beta_{42}$ and elevated MCBP for PIB identify presumptive preclinical AD in a substantial number of cognitively normal older adults and provide the opportunity to identify these individuals for longitudinal studies to determine their risk for DAT.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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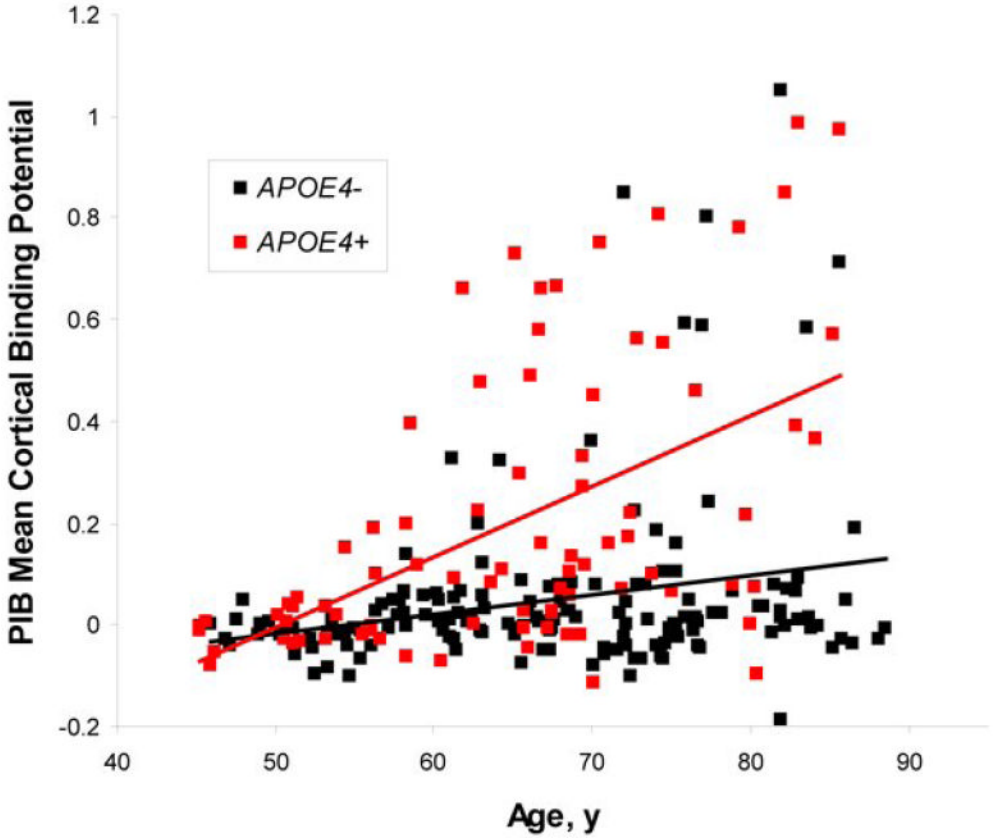


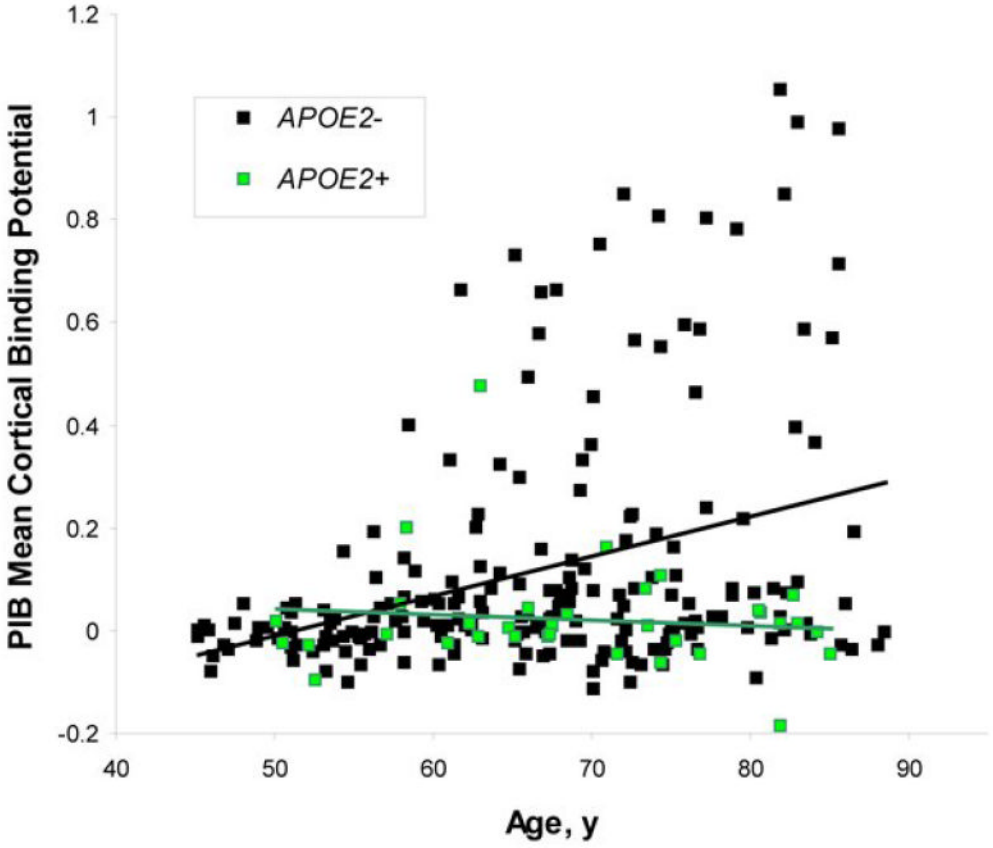
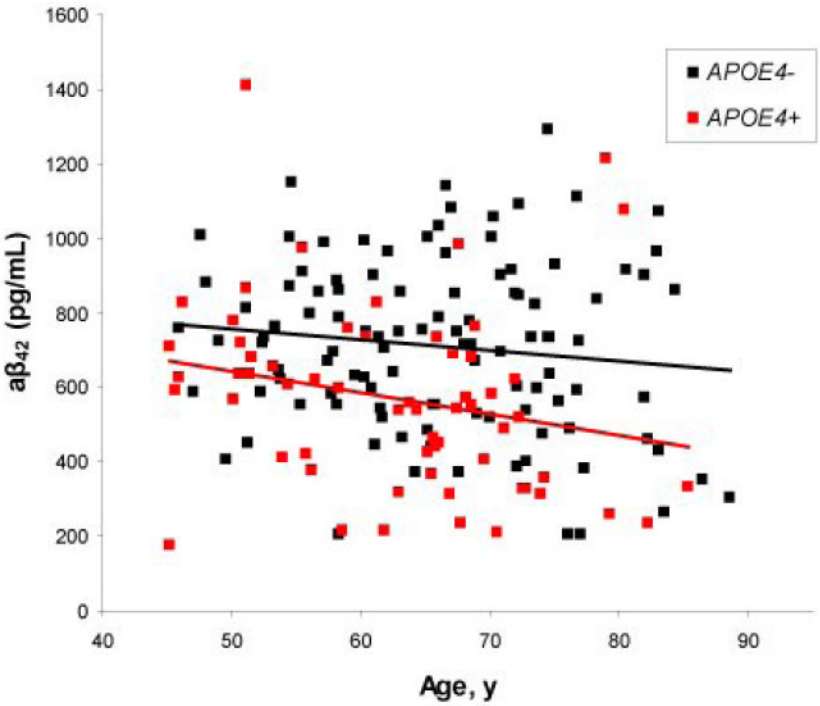
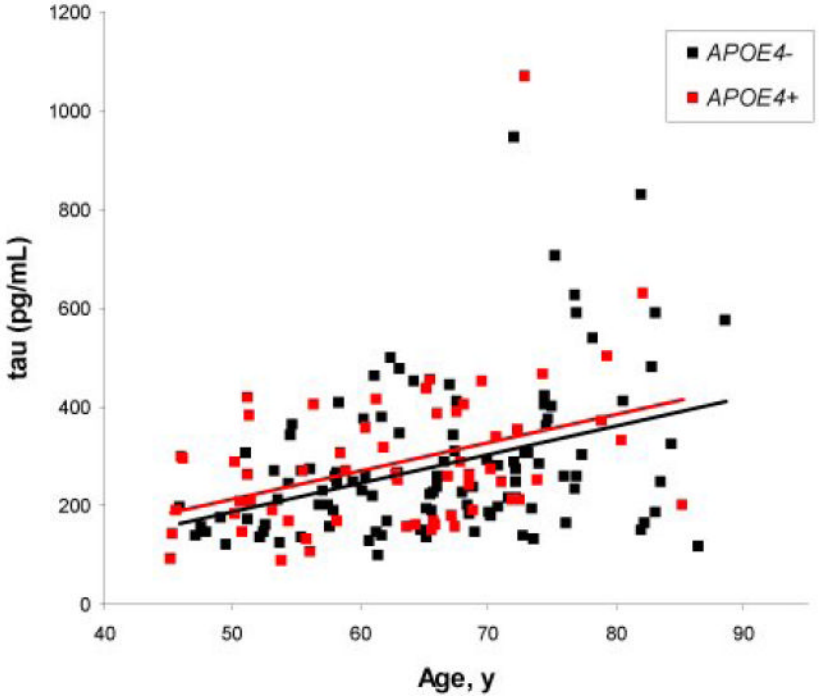


Figure 1.
Mean cortical binding potentials for Pittsburgh Compound-B (PIB) in 241 cognitively normal participants as a function of age at clinical assessment and of (A.) the presence (red squares) or absence (black squares) of the ε4 allele of Apolipoprotein E (*APOE*) or (B.) the presence (green squares) or absence (black squares) of the ε2 allele of *APOE*.





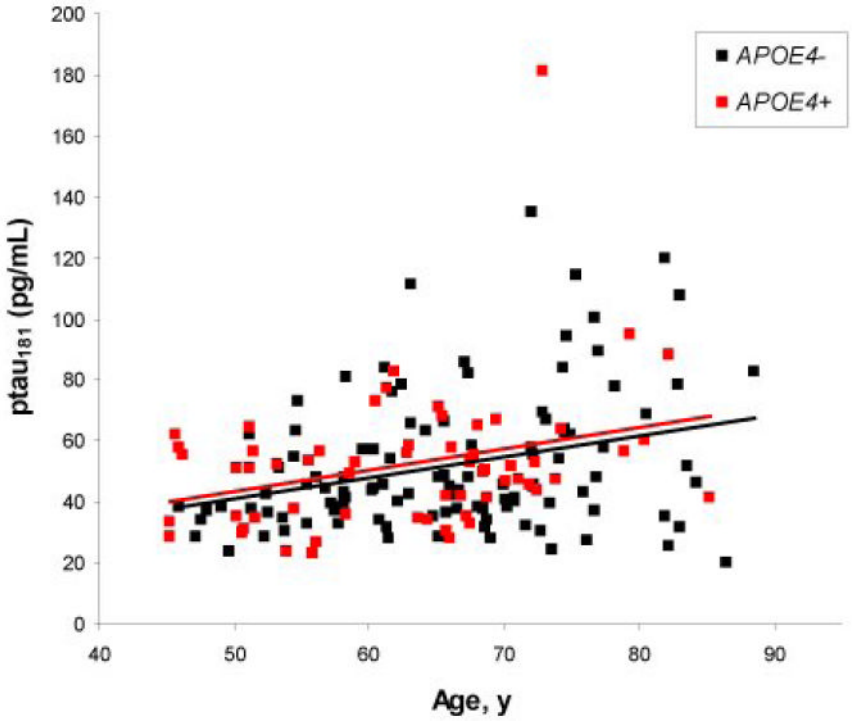
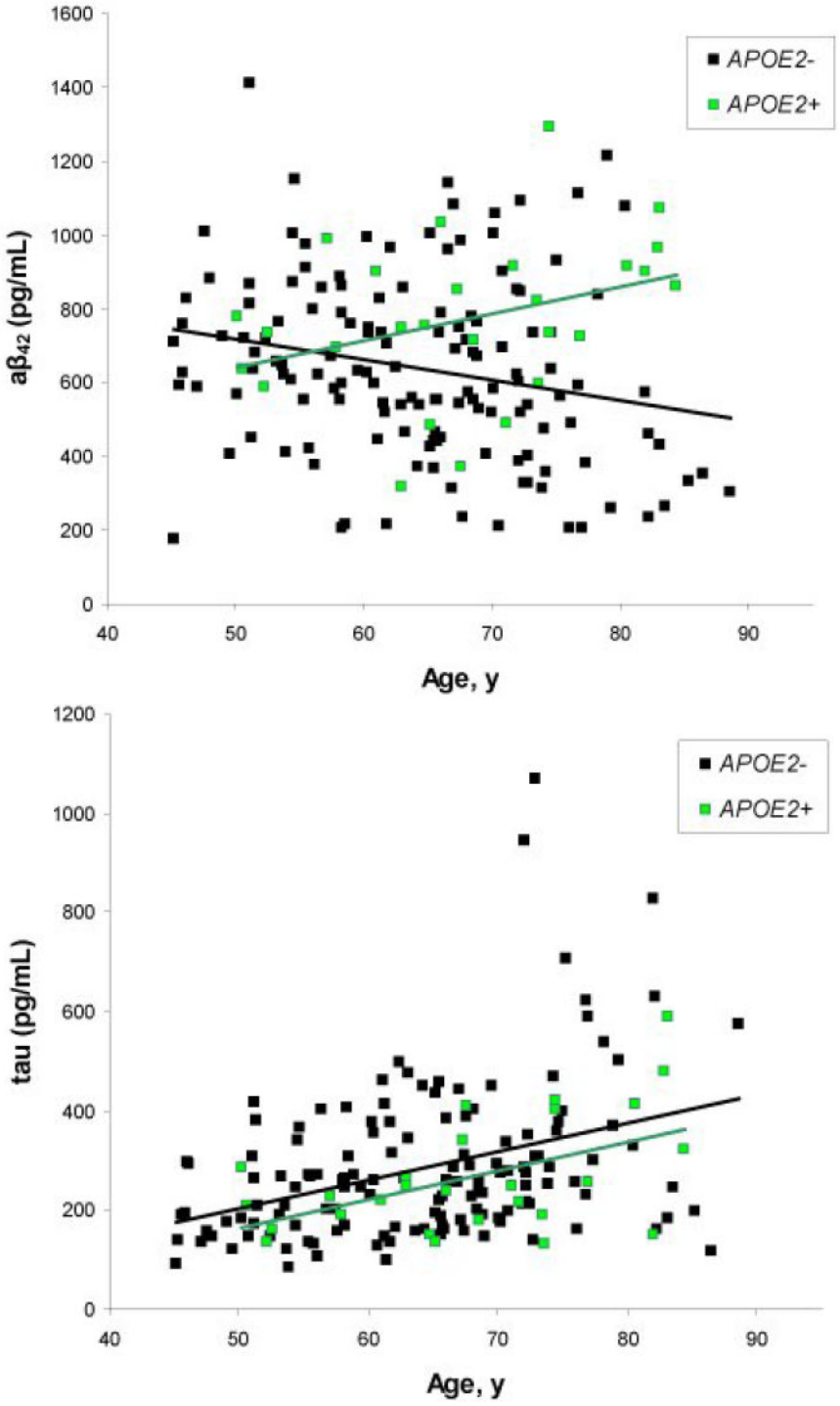


Figure 2.
Unadjusted associations with age at clinical assessment and the presence (red squares) or absence (black squares) of *APOE4* in 168 cognitively normal participants for cerebrospinal fluid (CSF) measures of: A. amyloid-beta42 ($A\beta_{42}$); B. tau; and C. tau phosphorylated at the threonine 181 position ($ptau_{181}$).



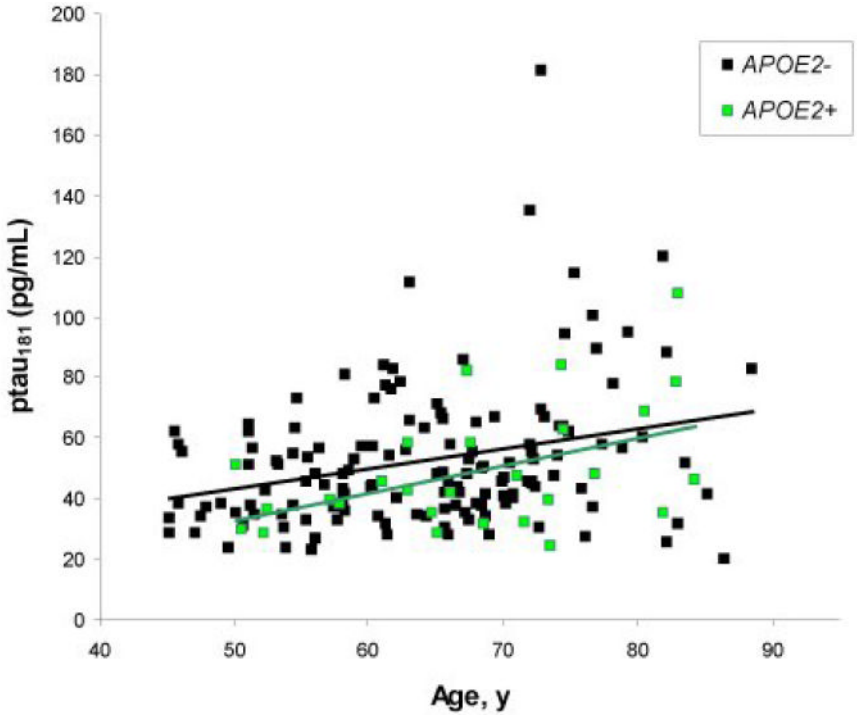


Figure 3.
Unadjusted associations with age at clinical assessment and the presence (green squares) or absence (black squares) of *APOE2* in the CSF of 168 cognitively normal individuals for CSF measures of A. $A\beta_{42}$; B. tau; and C. $ptau_{181}$.

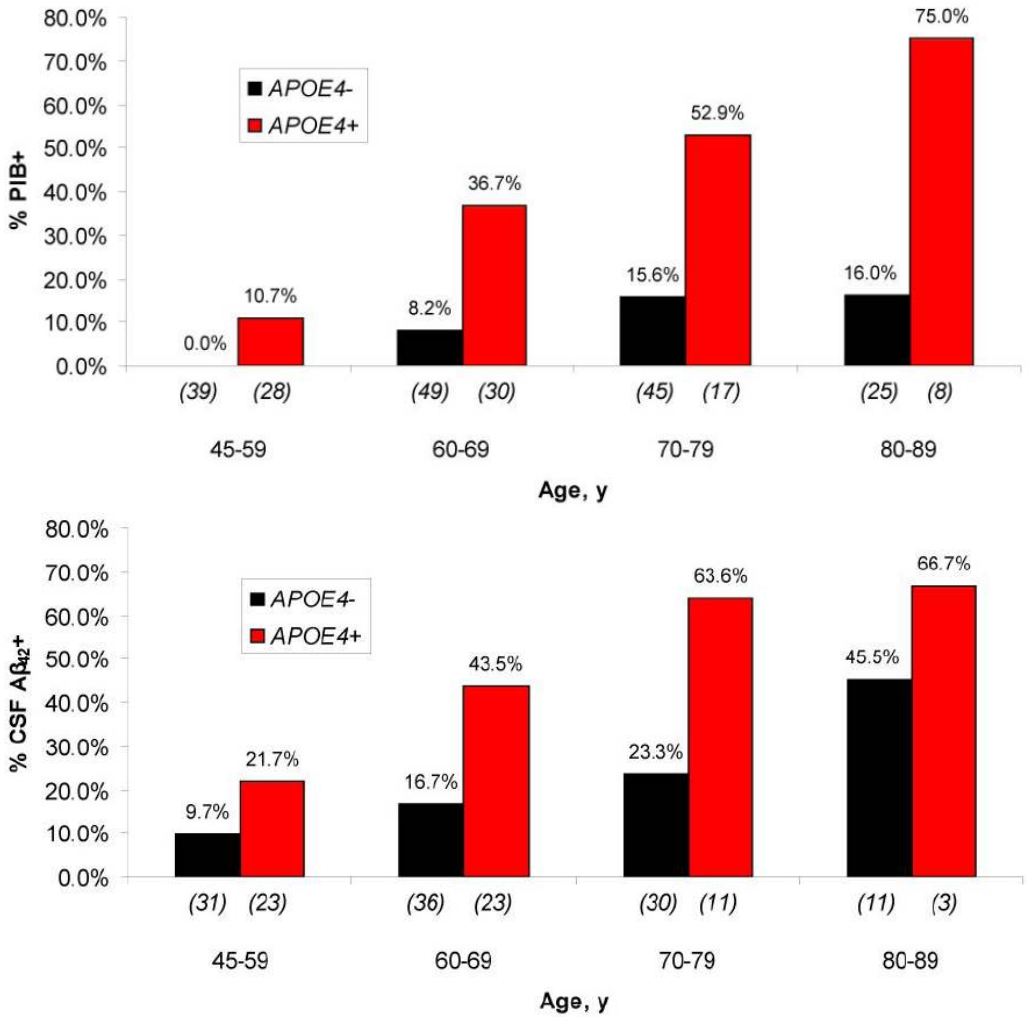


Figure 4.

A. Frequency by age group for individuals with MCBP for PIB >0.18 ("PIB-positive") in 241 cognitively normal individuals as a function of the presence (red bars) or absence (black bars) of *APOE4*.

B. Frequency by age group for individuals with CSF $A\beta_{42}$ <500pg/mL ("CSF $A\beta_{42}$ positive") in 168 cognitively normal individuals as a function of the presence (red bars) or absence (black bars) of *APOE4*.

Table 1

Characteristics of 241 nondemented participants.

	Mean/N	SD/%
Age (years)	66.8	10.7
Women (%)	164	68.1%
Race (%)		
European-American	215	89.2%
African-American	23	9.5%
Other	3	1.2%
Education (years)	15.9	2.7
MMSE	29.1	1.2
APOE genotype		
ε2/ε2	3	1.2%
ε2/ε3	26	10.8%
ε2/ε4	5	2.1%
ε3/ε3	129	53.5%
ε3/ε4	66	27.4%
ε4/ε4	12	5.0%

Legend: MMSE = Mini Mental State Examination, with possible range of scores from 30 (“best”) to 0 (“worst”). *APOE* is the gene encoding for apolipoprotein E.