Plasma Levels of Apolipoprotein E and Cognitive Function in Old Age

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ABSTRACT: The relationship between structural variants of the apolipoprotein E gene, APOE ε2/ε3/ε4, and dementia is well established, whereas the relationship of plasma apoE levels with dementia is less clear. Plasma apoE levels are under tight genetic control but vary widely within the various genotypes indicating that the APOE ε2/ε3/ε4 locus explains only a small fraction of this variation. Here we studied the association of plasma apolipoprotein E (apoE) levels with cognitive function in the elderly population at large. Within the Leiden 85-plus Study, a prospective population-based study of subjects aged 85 years, we measured plasma apoE level and genotype at base line. During a 5-year follow-up period, cognitive function was annually assessed using the Mini Mental State Examination (MMSE) and a standardized neuropsychological test battery. Among ε3ε3 carriers (n = 324), high plasma apoE levels associated with impaired global cognitive function (−1.10 points change in MMSE score per one standard deviation increase of plasma apoE level, P = 0.001), as well as lower attention (P = 0.064), speed and memory function (all P < 0.05). Adjustment for cardiovascular risk factors and exclusion of all subjects who suffered a stroke did not materially change the associations. Similar estimates were obtained in ε3ε4 carriers (n = 100), but not in ε2ε3 carriers (n = 90). We conclude that in old age, in non-ε2-allele carriers, high plasma apoE levels are associated with cognitive impairments, independent of genotype, cardiovascular risk factors, and stroke.

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INTRODUCTION

The relationship between structural variants of the apolipoprotein E gene, \( APOE \varepsilon2/\varepsilon3/\varepsilon4 \), and dementia is well established, whereas the relationship of plasma apolipoprotein (apoE) levels with dementia is less clear. Two \( APOE \) gene polymorphisms constitute the \( \varepsilon2/\varepsilon3/\varepsilon4 \)-alleles that encode three structurally different isoforms, apoE2, apoE3, and apoE4. Numerous studies have consistently shown that, compared to carriers of the \( APOE \varepsilon3 \)-allele, subjects with the \( \varepsilon4 \)-allele have an increased risk of developing dementia,\(^1\,^2\) whereas \( \varepsilon2 \)-allele carriers are at a lower risk.\(^3\)

The apoE2/3/4 isoforms have different affinity to receptors that regulate their clearance and as a result the isoforms circulate at different plasma levels.\(^4\) Plasma apoE levels are under tight genetic control\(^5\) but the \( APOE \) locus itself explains only a small fraction of this variation\(^6\) and plasma apoE levels thus vary within genotypes as well.\(^7\) Recently, we showed that, for each \( APOE \) genotype separately, plasma apoE levels strongly associate with an increased risk of mortality from cardiovascular causes.\(^8\) The association of plasma apoE levels with cognitive function in the elderly population at large has not been reported yet.

Here, we studied whether, for each \( APOE \) genotype separately, plasma apoE levels associate with cognitive function. Therefore, in the Leiden 85-plus Study, a population-based prospective follow-up study, we determined \( APOE \varepsilon2/\varepsilon3/\varepsilon4 \) genotypes, and plasma apoE levels at base line. Annual assessment of cognitive function was obtained over a follow-up period of 5 years.

MATERIALS AND METHODS

Participants

Between September 1, 1997 and September 1, 1999, a total of 705 inhabitants of the community of Leiden, The Netherlands, reached the age of 85 years. Among these 85-year-old persons, we initiated a follow-up study to investigate determinants of successful aging. There were no selection criteria on health or demographic characteristics. Fourteen inhabitants died before they could be enrolled. The response rate was 87%; a total of 599 subjects (397 women and 202 men) participated.\(^9\) There were no significant differences in various demographic characteristics between the 599 respondents and the source population. Of the 599 participants in the cohort, 38 refused to provide a blood sample, yielding a total number of 561 participants for this study. Subjects were visited within 1 month after their 85th birthday at their home for face-to-face interviews and neuropsychological testing. Subjects were revisited annually until
the age of 90 years. The Medical Ethical Committee of the Leiden University Medical Center approved the study, and informed consent was obtained from all subjects.

**APOE Genotypes**

For genotyping two TaqMan assays (Applied Biosystems, Foster City, CA) were used, that were described in detail elsewhere.\(^8\)

**Plasma Parameters**

Blood samples were collected early in the morning, although not fasting. Baseline plasma apoE levels were determined using a human apoE-specific sandwich enzyme-linked immunosorbent assay (ELISA) as described in detail elsewhere.\(^8\) Plasma levels of total cholesterol, HDL cholesterol, and triglycerides were analyzed at base line on fully automated computerized analyzers (Hitachi 747 and 911; Hitachi, Ltd., Tokyo, Japan). The level of LDL cholesterol was estimated by the Friedewald equation (LDL cholesterol [mmol/L] = total cholesterol–HDL cholesterol–[triglycerides/2.2]), whereby subjects with a triglycerides concentration higher than 443 mg/dL (5 mmol/L) were excluded (\(n = 5\)).

**Cognitive Function**

Global cognitive functioning was assessed with the Mini-Mental State Examination (MMSE).\(^{10}\) Possible scores on the MMSE range from 0 to 30 points, with lower scores indicating worse global cognitive functioning. Attention was measured using the third chart of the 40-item Stroop Test. Outcome was the time needed to name the ink color of 40 incongruous printed names of colors.\(^{11}\) Higher scores indicated worse attention. Processing speed was assessed with the Letter Digit Coding Test (LDT). Outcome was the total number of correct digits according to a code key in 60 s. Lower scores indicated a slower speed. Immediate and delayed recall memory was determined with the 12 Picture Learning Test (PLT-i and PLT-d). In this test, 12 pictures were presented, and the subject was asked to recall the presented pictures. This procedure was carried out three times. After 20 min, the participants were asked to recall the presented pictures again. Outcome for the immediate recall memory was the total number of correctly recalled pictures during the three procedures, and possible scores ranged from 0 to 36 pictures. Outcome for the delayed recall memory was the total number of correctly recalled pictures after 20 min, and
possible scores ranged from 0 to 12 pictures\textsuperscript{12}. Lower scores indicated worse memory. Parallel versions for the processing speed and memory tests, using identical procedures, but with different items were used to prevent learning effects. These five neuropsychological tests have been used in the assessment of large samples of elderly subjects and have been shown to be reliable and sensitive in the detection of small differences in cognitive function\textsuperscript{11,13}.

Global cognitive function was assessed in all participants. Attention, processing speed, and immediate and delayed recall memory were not administered in subjects with a MMSE score below 19 points because of a lack of reliability and validity of these tests in subjects with severe cognitive impairment. The percentage of participants with a MMSE score below 19 points increased from 17% at the age of 85 years (92 out of 546 participants) to 27% at the age of 90 years (72 out of 265 participants).

**Ischemic Cerebrovascular Disease**

The presence of stroke in the medical history and the incidence of stroke during the follow-up period were assessed by annually interviewing treating physicians of all subjects.

**Cardiovascular Risk Factors**

Subjects were classified as having diabetes when they met at least one of the following criteria: (a) history of type 2 diabetes obtained from the general practitioner or the subject’s treating physician; (b) use of sulfonylureas, biguanides, or insulin, based on information obtained from the subject’s pharmacist; or (c) nonfasting glucose of 11.1 mmol/L or higher. Subjects were classified as having hypertension when they met at least one of the following criteria: (a) history of hypertension obtained from the general practitioner or the subjects’ treating physician; or (b) mean systolic blood pressure of 165 mmHg or higher or diastolic blood pressure of 95 mmHg or higher. Of all subjects length and weight were measured at base line. Body mass index (BMI) was calculated from these measurements.

**Level of Education**

Level of education of each subject was determined by the number of years a subject went to school. Information was obtained at the first visit using a questionnaire. Low education was defined by $\leq 6$ years of schooling, whereas high education was defined by $\geq 7$ of schooling.
Statistical Analysis

Plasma levels of total and HDL and LDL cholesterol were distributed normally and are reported as means and standard deviations. Plasma levels of apoE and triglycerides were not normally distributed and are presented as medians and interquartile range to assess central tendency. The association between plasma apoE levels and the course of cognitive function during follow-up was analyzed with linear mixed models. The flexibility of mixed models makes them the preferred choice for the analysis of repeated measures data. Mixed models use all available data during follow-up, can properly account for correlation between repeated measurements, and can handle missing data more appropriately than traditional models. Plasma apoE levels were put in the model as a continuous variable, and dichotomized around the median. In an additional analysis, standard deviation scores of log-transformed plasma apoE levels were used. All models were adjusted for gender and level of education. Also, the association between plasma apoE levels and cognition was adjusted for cardiovascular risk factors using prevalence of hypertension and diabetes mellitus, BMI, and plasma levels of triglycerides, and HDL and LDL cholesterol as covariates in the linear mixed model. All calculations were performed using SPSS software (version 12.0.1, SPSS Inc., Chicago, IL).

RESULTS

From the 561 participants who were eligible for this study, APOE genotyping failed in 13 and measurement of plasma apoE level in 2 subjects. Figure 1 shows the annual follow-up visits of the 546 remaining participants from the age of 85 years through 90 years. Table 1 lists the baseline characteristics of the 546 participants. When all participants were analyzed, plasma apoE levels were inversely correlated with scores on the MMSE ($P < 0.001$). At base line subjects with plasma apoE levels above the median scored 1.29 points lower ($P = 0.015$) on the MMSE compared to those with plasma apoE levels below the median. However, these crude analyses are confounded by the various apoE isoforms. Compared to mean plasma apoE levels of APOE ε3ε3 carriers (mean plasma apoE level: 56.8 mg/L; SD: 31.2), ε4 carriers had 8.2 mg/L ($P = 0.012$) lower plasma apoE levels, whereas ε2 carriers had 22.0 mg/L ($P = 0.001$) higher plasma apoE levels. As the ε4-allele carriers are at an increased risk of cognitive impairment, whereas carriers of the ε2-allele are relatively protected, the correlation between plasma apoE levels and MMSE score is thus underestimated. When the analysis was adjusted for APOE genotypes, subjects with plasma apoE levels above the median scored 1.79 points lower ($P = 0.001$) on the MMSE when compared to those with plasma apoE levels below median.
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FIGURE 1. Annual follow-up visits of 546 participants from the age of 85 years through 90 years within the Leiden 85-plus Study.

To overcome confounding by the various isoforms we also analyzed the association between plasma apoE levels and cognitive function in groups of subjects with the same APOE ε2/ε3/ε4 genotype. These analyses were performed in the three largest genotype groups ε2ε3 (n = 90), ε3ε3 (n = 324), and ε3ε4 (n = 100). The genotype groups ε2ε2 (n = 4), ε2ε4 (n = 13), and ε4ε4 (n = 15) were considered too small to yield informative results. Figure 2 shows global cognitive function during the follow-up period from the age of 85 years through 90 years separately for those with plasma apoE levels above and below the median. Among ε2ε3 carriers, mean MMSE scores during follow-up were not significantly different between those with high and those with low plasma apoE levels ($P = 0.711$). Among ε3ε3- and ε3ε4 carriers, those with high plasma apoE levels had significantly lower mean MMSE scores during follow-up ($P = 0.001$ and $P = 0.018$, respectively).

When the association of plasma apoE levels and cognitive function was analyzed on a continuous scale, per standard deviation increase of log-transformed plasma apoE level, the MMSE score changed with −0.77 points ($P = 0.272$), −1.10 points ($P = 0.001$), and −2.32 points ($P = 0.001$) for carriers of the ε2ε3-, ε3ε3-, and ε3ε4 genotype, respectively. Adjustment for classical cardiovascular risk factors (plasma levels of lipids, BMI, prevalence of diabetes mellitus, and prevalence of hypertension) did not materially change the observed associations. The adjusted effect sizes were −1.06 points ($P = 0.190$) in ε2ε3 carriers, −1.45 points ($P < 0.001$) in ε3ε3 carriers, and −2.41 points ($P = 0.001$) in ε3ε4 carriers. When this analysis was repeated, now restricting it to only those subjects who did not have a history of stroke at the age of
TABLE 1. Baseline characteristics of the study participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number (n)</td>
<td>546</td>
</tr>
<tr>
<td>Men</td>
<td>181 (33%)</td>
</tr>
<tr>
<td>Institutionalized</td>
<td>97 (18%)</td>
</tr>
<tr>
<td>Low level of education&lt;sup&gt;a&lt;/sup&gt;</td>
<td>354 (65%)</td>
</tr>
<tr>
<td>History of stroke</td>
<td>55 (10%)</td>
</tr>
<tr>
<td>APOE genotype</td>
<td></td>
</tr>
<tr>
<td>ε2ε2</td>
<td>4 (1%)</td>
</tr>
<tr>
<td>ε2ε3</td>
<td>90 (17%)</td>
</tr>
<tr>
<td>ε2ε4</td>
<td>13 (2%)</td>
</tr>
<tr>
<td>ε3ε3</td>
<td>324 (59%)</td>
</tr>
<tr>
<td>ε3ε4</td>
<td>100 (18%)</td>
</tr>
<tr>
<td>ε4ε4</td>
<td>15 (3%)</td>
</tr>
<tr>
<td>Plasma apoE levels (in mg/L)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.2 (35.2–72.0)</td>
</tr>
<tr>
<td>Plasma lipid levels</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (in mmol/L)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.71 (1.13)</td>
</tr>
<tr>
<td>LDL cholesterol (in mmol/L)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.68 (0.98)</td>
</tr>
<tr>
<td>HDL cholesterol (in mmol/L)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.32 (0.40)</td>
</tr>
<tr>
<td>Triglycerides (in mmol/L)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.34 (1.01–1.95)</td>
</tr>
<tr>
<td>MMSE (points)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26 (22–28)</td>
</tr>
<tr>
<td>Stroop test (seconds)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>75 (60–98)</td>
</tr>
<tr>
<td>Letter digit coding test (digits)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16 (12–21)</td>
</tr>
<tr>
<td>Picture learning test immediate recall (pictures)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25 (20–28)</td>
</tr>
<tr>
<td>Picture learning test delayed recall (pictures)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9 (7–11)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Level of education was dichotomized by 6 years of schooling.
<sup>b</sup>Not normally distributed continuous data are presented as medians with interquartile ranges.
<sup>c</sup>Normally distributed continuous data are presented as means with standard deviation.

85 years and who did not suffer a stroke during the 5-year follow-up, the effect sizes were −0.09 points (P = 0.897), −1.12 points (P = 0.002), and −2.06 points (P = 0.007), respectively.

To investigate the association of plasma apoE levels and cognitive function in more detail we analyzed data from an additional cognitive test battery including the domains of attention, processing speed, and memory (immediate and delayed). These additional tests were not performed in subjects with a MMSE score below 19 points. TABLE 2 shows that in ε2ε3 carriers higher plasma apoE levels did not associate with worse performance on the cognitive tests. In ε3ε3 carriers, an increase in plasma apoE level associated with worse performance in attention (P = 0.064), processing speed (P = 0.033), immediate recall memory (P = 0.013), and delayed recall memory (P = 0.015). In ε3ε4 carriers similar trends were observed in the scores for attention, but not for processing speed, whereas the effect size on the memory tests was more pronounced. Estimates remained unchanged after adjustment for cardiovascular risk factors.
FIGURE 2. MMSE scores during 5-year follow-up dependent on plasma apoE levels in subjects with the APOE ε2ε3, ε3ε3, and ε3ε4 genotype. Plasma apoE levels were dichotomized around the median within each genotype group. Dots represent estimated mean (and standard error) scores using linear mixed models adjusted for sex, and level of education. Number of subjects present at the age of 90 years for each genotype in the group with low and the group with high plasma apoE levels, respectively: ε2ε3: 27, 25; ε3ε3: 101, 61; ε3ε4: 25, 18.
<table>
<thead>
<tr>
<th>Genotype</th>
<th>N</th>
<th>Stroop (seconds needed to perform task)</th>
<th>Change in test score per one standard deviation rise in plasma apoE level (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>LDT (number of correctly coded letters)</td>
</tr>
<tr>
<td>ε2ε3</td>
<td>83</td>
<td>+1.18 (3.37)</td>
<td>0.728</td>
</tr>
<tr>
<td>ε3ε3</td>
<td>278</td>
<td>+3.22 (1.73)</td>
<td>0.064</td>
</tr>
<tr>
<td>ε3ε4</td>
<td>75</td>
<td>−1.15 (4.32)</td>
<td>0.791</td>
</tr>
</tbody>
</table>

**NOTE.** Estimates represent the change in test score per standard deviation increase in log-transformed plasma apoE level. Higher test scores on Stroop and lower test scores on LDT, PLT-i, and PLT-d represent worse cognitive function. Change in test scores (and standard error) was calculated using linear mixed models adjusted for sex, and level of education.
DISCUSSION

The main finding of our study is that in old age, in non-ε2-allele carriers, high plasma apoE levels are associated with lower cognitive function, independent of plasma levels of lipids and other cardiovascular risk factors. Clinical strokes explained only a small proportion of the lower cognitive function during follow-up.

Our study is the first prospective study on the relation between plasma apoE levels and cognition that has been performed in the population at large. The association of plasma apoE levels with cognitive decline has already been studied providing inconclusive results. In the apoEurope Study\textsuperscript{15} and the Rotterdam Study\textsuperscript{16} subjects with Alzheimer’s disease (AD) had lower plasma apoE levels compared to control subjects. When adjusted for \textit{APOE} genotype, age, and gender this effect remained the same in the apoEurope Study, but disappeared largely in the Rotterdam Study. In contrast, results from a study by Taddei \textit{et al.} showed higher plasma apoE levels in subjects with AD compared to control subjects in both ε4- and non-ε4 carriers.\textsuperscript{17} Yet two other studies, performed by Scacchi \textit{et al.} and Folin \textit{et al.} showed no association of plasma apoE levels with AD.\textsuperscript{18,19} There are a number of possible explanations for these variable outcomes. First, these studies did not consistently stratify their analyses for genotype groups, thereby allowing the distorting effect of the isoforms of apoE. Second, in these studies cases were AD patients, representing a group of subjects with moderate to severely impaired cognitive function, thereby leaving out subjects with minor impaired cognitive function. As in this population-based study we have included individuals with a wide variety of cognitive function, it gave us the opportunity to detect more subtle differences in cognitive function.

Within the population under study, plasma apoE levels have been shown to associate strongly with cardiovascular mortality,\textsuperscript{8} suggesting that high plasma apoE levels associate with vascular pathologies. In the brain, strokes occur as the consequence of cardiovascular disease and contribute to poor cognition. To rule out the possible influencing effect of strokes we analyzed the data with the exclusion of all clinical strokes. This only partly explained a small part of the observed association. There are a number of possible mechanisms how plasma apoE levels, which are genetically determined, may affect cognition other than stroke. First, plasma apoE may cause vascular pathologies \textit{in cerebro} other than stroke that contribute to cognitive decline in old age. In autopsies, vascular pathologies have been observed in a large portion of brains and associated with cognitive function.\textsuperscript{20} These vascular pathologies included hemorrhages, infarctions, and lacunes as well as small vessel disease. Second, proinflammatory effects of systemic apoE may inflict damage in the brain. Recently, apoE has been shown to stimulate systemic immune responses by mediating lipid antigen presentation after binding and internalization by antigen-presenting cells through the LDL receptor.\textsuperscript{21} Peripheral cytokines, produced by the
proinflammatory effect of apoE, have been shown to penetrate the blood–brain barrier\textsuperscript{22} and considerable evidence has accumulated that inflammation plays a pivotal role in AD.\textsuperscript{23} Third, systemically produced apoE may cross the blood–brain barrier and inflict damage to the brain locally. It has been shown that cerebral apoE is locally produced by astrocytes and that cerebrospinal fluid levels of apoE do not correlate with plasma apoE levels, implying that systemically produced apoE does not cross the blood–brain barrier.\textsuperscript{24,25} However, others have shown that the blood–brain barrier is prone to change both structurally and functionally with increasing age\textsuperscript{26} and it has been shown that subjects with dementia have a blood–brain barrier dysfunction.\textsuperscript{27} Therefore, systemically produced apoE may cross the dysfunctional blood–brain barrier and interfere with pathological processes \textit{in cerebro} that lead to cognitive decline. For instance, it has been shown that apoE facilitates $\beta$-amyloid deposition in the brain in mouse models.\textsuperscript{28–30}

In subjects with the APOE $\varepsilon 2\varepsilon 3$ genotype high plasma apoE levels did not associate with an impaired cognitive function. ApoE2 has far lower affinity for LDL receptors compared to apoE3 and apoE4,\textsuperscript{31} and apoE exerts its proinflammatory effect and the regulation of the lipid metabolism, at least in part, through the LDL receptor. The effect of apoE2 in lipid antigen presentation was also lower when compared to apoE3.\textsuperscript{21} Taken together, these observations suggest a genotype specific effect of plasma apoE levels, which adds to the reasoning that high levels of plasma apoE3 and apoE4 are causal to cognitive decline, and not an epiphenomenon of other mechanisms.

A possible limitation of our study is that this finding cannot directly be extrapolated beyond this age group, especially because cognitive decline is an age-specific disorder. However, our study population of 85-year-old subjects represents 15\% of the men and 36\% of the women from the birth cohort 1912–1914 (official data from the Dutch Bureau of Statistics, www.cbs.nl). Apparently, a substantial portion of the total population reaches this age category and an even larger proportion will reach it in the future. Another possible limitation is that mortality of subjects in this study is dependent on plasma apoE levels, whereas the linear mixed models assume the missing of data to be independent of the determinant. However, mortality is higher in subjects with high plasma apoE levels, who also have a lower cognitive function. This may have resulted in an underestimation of cognitive decline in subjects with high plasma apoE levels. A third possible limitation is that the observed number of clinical strokes may have been an underestimation of the total number of cerebrovascular accidents. Minor strokes may not have been recognized as such by participants and health care workers. Imaging of the brain, such as magnetic resonance imaging (MRI), could help to identify more subclinical strokes, but are not available in this population-based study. As the effect of excluding the clinical, and thus the most severe, strokes was minimal, we favor the hypothesis that cerebrovascular accidents, including those that may not have been
detected clinically, cannot completely explain for the observed lower cognitive function.

A strong point of our study is that the data come from a population-based study without inclusion criteria on health and demographics. Therefore our results can be extrapolated to other populations in the same age range. Another strong point is that numbers were large, allowing stratification for the three largest genotype groups, thereby eliminating the potentially distorting effect of structural changes in the three isoforms of apoE. A third strong point is that the used neuropsychological tests have proven to be highly sensitive in the detection of small differences in cognitive function in elderly populations.\textsuperscript{11,32,33} Scores on these tests can be considered a marker of brain function and similarly a marker of anatomical and functional changes in the aging brains of the population under study.

In conclusion, we found that, in non-ε2-allele carriers, high plasma apoE levels are associated with lower cognitive function, independent of genotype, plasma levels of lipids, and other cardiovascular risk factors. This finding suggests that apart from the well-accepted association of apoE structural variation with cognitive function, apoE quantitative variation is an important and independent indicator of cognitive decline in old age.

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