Association Between Apolipoprotein E4 and Cognitive Decline in Elderly Adults

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OBJECTIVE: To determine the influence of apolipoprotein E on cognitive decline in a cohort of elderly men and women.

DESIGN: Prospective study.

SETTING: Scotland, Ireland, and the Netherlands.

PARTICIPANTS: Five thousand eight hundred four subjects aged 70 to 82 from the Prospective Study of Pravastatin in the Elderly at Risk (PROSPER).

MEASUREMENTS: Subjects were assessed at baseline and over a mean 3.2-year (range 0.7–4.2) follow-up for memory (Picture-Word Recall), speed of information processing (Stroop, and Letter-Digit Coding), global cognitive function (Mini-Mental State Examination), and activities of daily living.

RESULTS: At baseline, subjects with apolipoprotein E4 versus those without E4 had poorer memory performance (mean score difference $-0.20$ (95% confidence interval $[-0.31$ to $-0.09]$) for immediate recall and $-0.32$ (95% CI $=-0.48$ to $-0.16$) for delayed recall and slower information processing (difference in Stroop, 2.79 seconds, (95% CI $=1.20$–$4.28$); Letter-Digit score, $-0.36$, (95% CI $=-0.77$–$0.05$). Subjects with apolipoprotein E4 showed a greater decline in immediate ($-0.22$, 95% CI $=-0.33$ to $-0.11$) and delayed ($-0.30$, 95% CI $=-0.46$ to $-0.15$) memory scores but no significant change in speed of information processing (Stroop, $P=.17$; Letter-Digit, $P=.06$). Memory scores decreased 2.5% from baseline in those without E4, 4.3% in E4 heterozygotes ($P=.01$ for immediate and $P=.03$ for delayed, vs no E4) and 8.9% to 13.8% in E4 homozygotes ($P=.04$ for immediate and $P=.004$ for delayed, vs heterozygotes). Apolipoprotein E4 was associated with greater decline in instrumental activities of daily living ($P<.001$). Cognitive decline was not associated with lipoprotein levels.


Key words: memory; dementia; trial; statin

A major challenge in the quest to promote healthy aging is to uncover the determinants of cognitive decline in the general population and the risk factors that lead to the development of frank dementia. Although age is the main predictor of cognitive function, investigators have also reported that a history of hypertension, diabetes mellitus, stroke, depression, and lack of physical activity are factors.1–6 The apolipoprotein E phenotype (apolipoprotein E is coded by a gene (e) that exhibits allelic variants e2, e3, and e4) has been linked consistently and strongly to the appearance of Alzheimer’s disease and to some aspects of cognitive decline in elderly cohorts.1,2,5–9 Inheritance of E4 (the product of e4) especially in the homozygous form, has been associated in cross-sectional studies with risk of Alzheimer’s disease and poorer global cognitive function, episodic memory, and executive function, although the magnitude of the effect is modest.10,11 Longitudinal data also show good evidence of an association between E4 and risk of Alzheimer’s disease and dementia,12,13 although the rela-
tionship between the E4 genotype and age-associated cognitive decline without dementia has been less clear. One study found a link in twins between E4 and deterioration of working memory, and others have reported greater decline in verbal memory and executive function in those with E4. In contrast, another study found that possession of E4 did not modify progression of cognitive decline in the preclinical period of Alzheimer's disease, and a third study reported slower rates of memory decline in E4 carriers. Likewise, it was found that E4 did not contribute to prediction of cognitive decline in the very old but that as of the time of writing, the role of apolipoprotein E in brain physiology and the precise nature of the influence of the phenotypic variation on the pathogenesis of neurodegenerative disease is unknown, although clues are emerging from molecular studies and from investigations of white matter structure.

The nature of the association between lipid and lipoprotein levels and cognitive impairment is largely unknown, with conflicting results being reported. Recently, low high-density lipoprotein cholesterol (HDL-C) levels have been linked to poorer cognitive function, independent of an effect on cardiovascular disease in the oldest old, whereas no association between total plasma cholesterol or HDL-C and risk of Alzheimer's disease was observed in another study.

The Prospective Study of Pravastatin in the Elderly at Risk (PROSPER) was a trial of statin use for the prevention of cardiovascular and cerebrovascular disease in 5,804 men and women aged 70 to 82. It demonstrated that pravastatin treatment was associated with a reduction in vascular events. Part of the trial design was to examine the effect of statin therapy on cognition. To this end, a battery of cognitive function tests was administered at baseline and annually during follow-up and linked to initial lipid levels, apolipoprotein E phenotype, and treatment allocation. It was reported previously that pravastatin use did not affect cognitive decline. Here, the effect of apolipoprotein E phenotype and lipoprotein levels on cognitive function at baseline and on cognitive decline are described in this large cohort. On the basis of previous work in smaller studies and the role of vascular disease in dementia, the working hypothesis was that apolipoprotein E phenotype and levels of low-density lipoprotein cholesterol (LDL-C) and HDL-C would influence cognition.

METHODS

Study Design and Subjects

PROSPER was a trial of statin use in preventing coronary and cerebrovascular events in older subjects with a history of vascular disease or at high risk of an event due to a history of smoking, hypertension, or diabetes mellitus. A total of 5,804 individuals (2,804 men, 3,000 women; aged 70–82) were recruited in Scotland, Ireland, and the Netherlands and randomized to receive 40 mg/d pravastatin or placebo daily. Follow-up was for 3.2 years on average (range 0.7–4.2 years).

As part of the design of the study, whether statin treatment had any effect on cognitive decline was assessed by administering a battery of cognitive function tests. Determinants of cognitive function at baseline and of cognitive decline were sought from the risk factor profiles, including apolipoprotein E phenotype, which was assessed during screening. There were restrictions on entry; plasma cholesterol had to be in the range of 4.0 to 9.0 mmol/L and plasma triglyceride less than 6.0 mmol/L. The institutional ethics review boards of the centers approved the study, and all participants gave written informed consent.

Measurements

Plasma cholesterol, triglyceride, LDL-C, and HDL-C were measured twice at fasting visits during the placebo run-in phase according to the Lipid Research Clinics protocol in a central laboratory that was standardized through the Centers for Disease Control and Prevention network. Apolipoprotein E phenotype was determined on plasma samples using Western blotting following a method previously established. Subjects were classified according to the presence of the E2, E3, or E4 bands on gel blots. The gel phenotyping method shows high concordance (>95%) with genotype testing according to allele-specific oligonucleotide assay (unpublished results).

A detailed description of the cognitive tests used in the study has been published previously. The Mini-Mental State Examination (MMSE) is used widely to screen for cognitive impairment and dementia. A cutoff score of 24 points or more (out of 30) was used as an inclusion criterion to eliminate those with poor cognitive function at baseline. Memory was tested using the Picture-Word Recall test based on the Groningen-Fifteen Words test. This measures recall, both immediate and after 20 minutes, of 15 pictures (rather than words, to overcome any language problem). The outcome variable is the mean number of correctly recalled pictures over three immediate trials and number recalled after the delay. Attention and processing were assessed using the Stroop-Color Word test and the Letter-Digit Coding test. The former, in the key Part III of the test, presents color names printed in incongruously colored ink (e.g., the word green printed in blue ink). Performance, timed in seconds to complete the test, measures the ability to discard the irrelevant name (green) in favor of the color of the ink (blue). The latter asks the subject to fill in digits next to letters according to a key; the outcome is the number of correct entries in 60 seconds. Subjects were assessed twice (2 weeks apart) at baseline to allow for any training effect. The results of the second test were taken as the starting estimate of cognitive function. Decline in basic activities of daily living (ADLs) was assessed using the 20-point variation of the Barthel Index and in extended activities instrumental activities of daily living (IADLs) using a 14-point score. All tests were repeated at 9, 18, and 30 months and at the final trial visit. Dementia was recorded as an adverse event if diagnosed by an attending physician; no trial-specific assessment was performed, and it was not an adjudicated endpoint.

Statistical Analyses

Subjects were divided initially into three categories (E4+ (phenotypes E3/4, E2/4, and E4/4), E3/3 (the common-
Influence of Apolipoprotein E Phenotype
Apolipoprotein E phenotype had a significant association with a number of tests of cognitive function, determined upon entry into the study (Table 1). As discussed in the Methods section, none of the E2+ versus E3/3 comparisons indicated a significant difference, and these phenotypes were combined in the E4− category. Subjects in the E4+ group performed significantly less well on the Stroop test and Picture-Word Recall (immediate and delayed) than subjects in the E4− group and had marginally, but significantly, poorer scores on the MMSE (Table 1). A significant influence of apolipoprotein E status was seen for the Letter-Digit Coding test or delay in memory function (i.e., immediate and delayed Picture-Word Recall). History of vascular disease, history of stroke, body mass index, diabetes mellitus, and smoking were predictors of scores in the Stroop and Picture-Word Recall. History of vascular disease, history of stroke, body mass index, diabetes mellitus, and smoking were predictors of scores in the Stroop and Picture-Word Recall.

RESULTS
Of the 5,804 subjects recruited to the study, apolipoprotein E phenotyping was available for 95.5%; 38 (0.7%) were E222, 621 (11.2%) were E223, 119 (2.2%) were E244, 3,496 (63.1%) were E233, 1,169 (21.1%) were E334, and 101 (1.8%) were E444. Translated into genotypes, the frequencies were in Hardy-Weinberg equilibrium. Thus, a total of 4,155 subjects were classified as E4− and 1,389 as E4+. In univariate analysis, the latter group had higher plasma total cholesterol (mean ± standard deviation 5.84 ± 0.91 vs. 5.63 ± 0.90 mmol/L, P < 0.001), higher LDL-C (3.96 ± 0.81 vs. 3.74 ± 0.79 mmol/L, P < 0.001), lower HDL-C (1.25 ± 0.34 vs. 1.29 ± 0.35 mmol/L, P < 0.001), and higher plasma triglyceride (1.60 ± 0.75 vs. 1.52 ± 0.68 mmol/L, P < 0.001) levels than the former. No significant difference was observed in blood pressure; sex; history of coronary disease, stroke, or hypertension; smoking habit; years of education; or a composite score of stroke risk. Subjects with diabetes mellitus were approximately 25% less prevalent in the E4+ group (P = 0.007). Subjects who were E4+ were marginally younger than those who were E4−.

At baseline, age, sex, and educational status were major determinants of all tests of cognitive function. History of stroke, diabetes mellitus, and smoking were predictors of scores in the Stroop and Letter-Digit Coding tests but not of memory function (i.e., immediate and delayed Picture-Word Recall). History of vascular disease, history of stroke, body mass index, diabetes mellitus, and smoking were predictors of scores in the Barthel and IADL indices (data not shown).

Repeatea measures models that included all of the measurements recorded on a subject and incorporated a linear separation between the groups were investigated, although these more-complex models did not add statistical power, possibly because of the additional assumptions made being invalid. This approach is not reported here; rather the simpler change from baseline analysis was used.

To examine the association between cognition and LDL-C and HDL-C, linear models were constructed as for apolipoprotein E analysis. For descriptive purposes, least squares linear models were constructed as for apolipoo protein E analysis. For descriptive purposes, least squares linear models were constructed as for apolipoprotein E analysis.

For all analyses, only complete and reliable test results of cognitive function (as indicated by the study nurse who administered the test) were included.
end of the study, 85 (6.4%) in the E₄⁺ group and 131 (3.3%) in the E₄⁻ group had scores less than 24 on the MMSE (P < .001 for difference in incidence), and there were 37 cases of dementia reported as an adverse event (2.7%) in the E₄⁺ group and 46 (1.1%) in the E₄⁻ group, a 2.48-fold difference in risk (P < .001). Including only those with MMSE scores of 26 to 30 at baseline produced the same qualitative result (data not shown).

Treatment assignment (i.e., to placebo or pravastatin therapy) was included in the multivariate models. It was reported previously that pravastatin use during the trial had no significant effect on change in cognitive function.42 This was further tested by examining the interaction between apolipoprotein E phenotype, treatment, and cognition. No significant associations were found with any of the tests, confirming the lack of effect of statin therapy on cognitive decline (data not shown).

Table 1. Comparison of Baseline Measures of Cognition According to Apolipoprotein E Phenotype

<table>
<thead>
<tr>
<th>Cognitive Test and Apolipoprotein E Phenotype</th>
<th>Baseline, Least Square Mean (Standard Error)</th>
<th>E₄⁺ versus E₄⁻ Mean Difference (95% Confidence Interval)</th>
<th>P-Value¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stroop Part III (seconds to complete) (n = 5,163)</td>
<td></td>
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<tr>
<td>E₄⁺</td>
<td>71.1 (0.93)</td>
<td>2.79 (1.30, –4.28)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>E₃/3</td>
<td>68.3 (0.77)</td>
<td>–0.36 (0.77, –0.05)</td>
<td>.08</td>
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<tr>
<td>E₂/+</td>
<td>68.1 (1.17)</td>
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<tr>
<td>Letter-Digit Coding (number correct) (n = 5,185)</td>
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<tr>
<td>E₄⁺</td>
<td>22.1 (0.26)</td>
<td>–0.36 (0.77, –0.05)</td>
<td>.08</td>
</tr>
<tr>
<td>E₃/3</td>
<td>22.5 (0.21)</td>
<td></td>
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<tr>
<td>E₂/+</td>
<td>22.5 (0.32)</td>
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<tr>
<td>Picture-Word Recall (number recalled) (n = 5,222)</td>
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<tr>
<td>Immediate</td>
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<tr>
<td>E₄⁺</td>
<td>9.16 (0.07)</td>
<td>–0.20 (–0.31, –0.09)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>E₃/3</td>
<td>9.35 (0.06)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E₂/+</td>
<td>9.44 (0.09)</td>
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<tr>
<td>Delayed</td>
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<tr>
<td>E₄⁺</td>
<td>9.77 (0.10)</td>
<td>–0.32 (–0.48, –0.16)</td>
<td>&lt;.001</td>
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<tr>
<td>E₃/3</td>
<td>10.07 (0.08)</td>
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<tr>
<td>E₂/+</td>
<td>10.24 (0.12)</td>
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<tr>
<td>Barthe Index (score) (n = 5,544)</td>
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<tr>
<td>E₄⁺</td>
<td>19.6 (0.03)</td>
<td>–0.04 (–0.08, –0.00)</td>
<td>.08</td>
</tr>
<tr>
<td>E₃/3</td>
<td>19.6 (0.02)</td>
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<tr>
<td>E₂/+</td>
<td>19.7 (0.03)</td>
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<tr>
<td>Instrumental activity of daily living index (score) (n = 5,544)</td>
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<tr>
<td>E₄⁺</td>
<td>13.4 (0.04)</td>
<td>–0.05 (–0.11, –0.01)</td>
<td>.12</td>
</tr>
<tr>
<td>E₃/3</td>
<td>13.4 (0.03)</td>
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<tr>
<td>E₂/+</td>
<td>13.4 (0.05)</td>
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<tr>
<td>Mini-Mental State Examination (score) (n = 5,479)</td>
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<tr>
<td>E₄⁺</td>
<td>27.8 (0.06)</td>
<td>–0.21 (–0.30, –0.12)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>E₃/3</td>
<td>28.0 (0.05)</td>
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<tr>
<td>E₂/+</td>
<td>28.0 (0.07)</td>
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</tr>
</tbody>
</table>

* E₄⁺ includes E₄/4, E₄/3, and E₄/2 phenotypes, E₃/3 includes E₃/2 and E₃/1.
  † Adjusted for age, sex, country, education, history of vascular disease, history of myocardial infarction, history of diabetes mellitus, history of stroke or transient ischemic attack, smoking, use of antihypertensive medication, blood pressure, body mass index, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglyceride, and where applicable test version.
  ‡ Comparison of mean test results in E₄⁺ (E₄/4, E₄/2, E₄/3) subjects versus E₄⁻ (E₃/3, E₂/3, E₂/2) subjects.
  § Apolipoprotein E₄⁺ group result was significantly different from E₃/3 and E₂/+ result.

Table 2. Comparison of Change in Cognitive Function According to Apolipoprotein E Phenotype

<table>
<thead>
<tr>
<th>Cognitive Test and Apolipoprotein E Phenotype</th>
<th>Change, Least Square Mean (Standard Error)</th>
<th>E₄⁺ versus E₄⁻ Mean Difference (95% Confidence Interval)</th>
<th>P-Value¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stroop Part III (seconds to complete) (n = 4,897)</td>
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<td></td>
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<tr>
<td>E₄⁺</td>
<td>5.99 (0.85)</td>
<td>+0.95 (0.39–2.29)</td>
<td>.17</td>
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<tr>
<td>E₃/3</td>
<td>5.02 (0.70)</td>
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<tr>
<td>E₂/+</td>
<td>5.14 (1.06)</td>
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<tr>
<td>Letter-Digit Coding (number correct) (n = 4,953)</td>
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<tr>
<td>E₄⁺</td>
<td>1.88 (0.18)</td>
<td>–0.26 (0.53–0.01)</td>
<td>.06</td>
</tr>
<tr>
<td>E₃/3</td>
<td>1.60 (0.14)</td>
<td></td>
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<tr>
<td>E₂/+</td>
<td>1.77 (0.21)</td>
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<tr>
<td>Picture-Word Recall (number recalled) (n = 5,004)</td>
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<tr>
<td>Immediate</td>
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</tr>
<tr>
<td>E₄⁺</td>
<td>0.52 (0.068)</td>
<td>–0.22 (0.33, –0.11)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>E₃/3</td>
<td>0.30 (0.056)</td>
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<tr>
<td>E₂/+</td>
<td>0.31 (0.085)</td>
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<tr>
<td>Delayed</td>
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<tr>
<td>E₄⁺</td>
<td>0.82 (0.098)</td>
<td>–0.30 (0.46, –0.15)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>E₃/3</td>
<td>0.51 (0.080)</td>
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<tr>
<td>E₂/+</td>
<td>0.58 (0.121)</td>
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<tr>
<td>Mini-Mental State Examination (score) (n = 5,260)</td>
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<tr>
<td>E₄⁺</td>
<td>0.63 (0.068)</td>
<td>–0.13 (0.24, –0.02)</td>
<td>.02</td>
</tr>
<tr>
<td>E₃/3</td>
<td>0.51 (0.056)</td>
<td></td>
<td></td>
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<tr>
<td>E₂/+</td>
<td>0.48 (0.086)</td>
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</tbody>
</table>

* E₄⁺ includes E₄/4, E₄/3, E₄/2; E₃/3 includes E₃/3, E₂/2.
  † Change score (last recorded result minus baseline result) adjusted for age, sex, country, education, history of vascular disease, history of myocardial infarction, history of diabetes mellitus, history of stroke or transient ischemic attack, smoking, use of antihypertensive medication, blood pressure, body mass index, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglyceride, and where applicable test version plus baseline test score and treatment allocation.
  ‡ Comparison of mean change in E₄⁺ and E₄⁻ groups.
  § A positive change in the Stroop test indicates deteriorating performance.
  ¶ Apolipoprotein E₄⁺ group result was significantly different from E₃/3 result.
  # Apolipoprotein E₄⁺ group result was significantly different from E₂/+ result.
Table 3. Apolipoprotein E4 Status and Cognitive Function

<table>
<thead>
<tr>
<th>Cognitive Test</th>
<th>E4 – (N = 4,155)*</th>
<th>E4 Heterozygotes (N = 1,288)*</th>
<th>E4 Homozygotes (N = 101)*</th>
<th>P-Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Least Square Mean (Standard Error)</td>
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<tr>
<td>Stroop Part III (seconds to complete)</td>
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</tr>
<tr>
<td>Baseline (n = 5,163)</td>
<td>68.3 (0.75)</td>
<td>71.1 (0.95)§</td>
<td>70.1 (2.52)</td>
<td>.001</td>
</tr>
<tr>
<td>Change (n = 4,897)</td>
<td>1.04 (0.68)</td>
<td>5.37 (0.68)</td>
<td>+9.42 (2.28)</td>
<td>.10</td>
</tr>
<tr>
<td>Letter-Digit Coding (number correct)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (n = 5,185)</td>
<td>22.5 (0.21)</td>
<td>22.2 (0.26)</td>
<td>21.4 (0.70)</td>
<td>.12</td>
</tr>
<tr>
<td>Change (n = 4,953)</td>
<td>1.63 (0.14)</td>
<td>1.85 (0.17)</td>
<td>2.41 (0.46)</td>
<td>.08</td>
</tr>
<tr>
<td>Picture-Word Recall (number recalled)</td>
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<tr>
<td>Immediate</td>
<td></td>
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</tr>
<tr>
<td>Baseline (n = 5,222)</td>
<td>9.36 (0.055)</td>
<td>9.18 (0.070)§</td>
<td>8.93 (0.185)§</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Change (n = 5,004)</td>
<td>0.00 (0.054)</td>
<td>0.26 (0.099)§</td>
<td>-1.00 (0.184)§</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Delayed</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Baseline (n = 5,222)</td>
<td>10.09 (0.079)</td>
<td>9.81 (0.100)§</td>
<td>9.33 (0.264)§</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Change (n = 5,004)</td>
<td>0.52 (0.078)</td>
<td>0.76 (0.099)§</td>
<td>1.72 (0.264)§</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Barthel Index (score)</td>
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<td></td>
</tr>
<tr>
<td>Baseline (n = 5,544)</td>
<td>19.6 (0.022)</td>
<td>19.6 (0.027)</td>
<td>19.6 (0.073)</td>
<td>.21</td>
</tr>
<tr>
<td>Change (n = 5,453)</td>
<td>0.51 (0.055)</td>
<td>0.63 (0.069)</td>
<td>-0.72 (0.185)</td>
<td>.06</td>
</tr>
<tr>
<td>Instrumental activities of daily living index (score)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (n = 5,544)</td>
<td>13.4 (0.030)</td>
<td>13.4 (0.038)</td>
<td>13.4 (0.101)</td>
<td>.30</td>
</tr>
<tr>
<td>Change (n = 5,454)</td>
<td>0.89 (0.066)</td>
<td>-1.21 (0.083)§</td>
<td>-1.18 (0.223)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Mini-Mental State Examination (score)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (n = 5,479)</td>
<td>28.0 (0.046)</td>
<td>27.8 (0.058)§</td>
<td>27.6 (0.153)§</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Change (n = 5,460)</td>
<td>-0.14 (0.058)</td>
<td>-0.37 (0.073)§</td>
<td>-1.11 (0.197)§</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

* E4 – includes E2/2, E2/3, E3/3; E4 heterozygotes include E4/3, E4/2; E4 homozygotes are E4/4. The number of subjects given is the maximum in each group. Data are adjusted for age, sex, country, education, history of vascular disease, history of myocardial infarction, history of diabetes mellitus, history of stroke or transient ischemic attack, smoking, use of antihypertensive medication, blood pressure, body mass index, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglyceride, and where applicable test version. Change score (last recorded result minus baseline result) was also adjusted for baseline test score and baseline test score and treatment allocation.

† P-value for test of heterogeneity across groups.

§ A positive change in the Stroop test indicates a deteriorating performance.

§ E4 heterozygotes or E4 homozygotes differ significantly from E4 –.

** E4 homozygotes differ significantly from E4 heterozygotes.

Barthel or IADL indices. MMSE scores of E4 homozygotes were significantly lower than those with E4 – (P = .004), but the comparison between the former and E4 heterozygotes was not significant (P = .11), although the means differed. Memory tests exhibited a trend toward stepwise reduction with E4 gene dosage. E4 homozygotes and heterozygotes had significantly lower scores than E4 –, although possibly because of small subgroup size, the mean scores of homozygotes while tending to be lower than those of heterozygotes, were not significantly different (P = .17 for immediate memory; P = .07 for delayed memory).

The decline in performance on the Stroop and Letter-Digit Coding tests was not significantly different in E4 –, E4 heterozygotes, and E4 homozygotes (Table 3). For the immediate and delayed memory tests, the decrement in mean score for the E4 homozygotes was significantly greater than that for the heterozygotes (P = .006 for immediate; P < .001 for delayed memory). In turn, the decline for E4 heterozygotes was greater than that for E4 – (P = .001 for immediate memory; P = .004 for delayed memory). A similar pattern was present for change in MMSE (P < .001 for E4 homozygotes vs heterozygotes; P < .001 for heterozygotes vs E4 –). To test the effect of baseline MMSE score on the association between apolipoprotein E phenotype and decline in memory function, an average percentage decline in the scores for the immediate and delayed Picture-Word recall was computed. This combined variable gave significant differences for E4 homozygotes, E4 heterozygotes, and E4 – subjects in the whole cohort (Figure 1) and the apolipoprotein E effect persisted when subjects were restricted to those with MMSE scores of 26 to 30. A similar pattern was seen in subjects with baseline MMSE scores of 28 to 30, with an overall significant effect of phenotype; intergroup comparisons were at the border of significance, probably because of smaller numbers of patients.

Influence of Lipoprotein Levels

Examination of the association between apolipoprotein E phenotype and decline in memory function, an average percentage decline in the scores for the immediate and delayed Picture-Word recall was computed. This combined variable gave significant differences for E4 homozygotes, E4 heterozygotes, and E4 – subjects in the whole cohort (Figure 1) and the apolipoprotein E effect persisted when subjects were restricted to those with MMSE scores of 26 to 30. A similar pattern was seen in subjects with baseline MMSE scores of 28 to 30, with an overall significant effect of phenotype; intergroup comparisons were at the border of significance, probably because of smaller numbers of patients.

Examination of the association between apolipoprotein E phenotype and decline in memory function, an average percentage decline in the scores for the immediate and delayed Picture-Word recall was computed. This combined variable gave significant differences for E4 homozygotes, E4 heterozygotes, and E4 – subjects in the whole cohort (Figure 1) and the apolipoprotein E effect persisted when subjects were restricted to those with MMSE scores of 26 to 30. A similar pattern was seen in subjects with baseline MMSE scores of 28 to 30, with an overall significant effect of phenotype; intergroup comparisons were at the border of significance, probably because of smaller numbers of patients.
scores being seen in the lowest LDL-C tertile, and the Stroop tests show data in the same direction. The Barthel and IADL indices were significantly related to LDL-C level, with lower scores again being seen in the lowest tertiles. No significant association was observed between these indices and HDL-C.

Lipoprotein concentrations at baseline had no significant effect on change in cognitive function or decrease in score on the MMSE or the Barthel or IADL index (Table 4).

DISCUSSION

This study of the determinants of cognition in elderly adults found a marked effect of apolipoprotein E phenotype on memory performance that was apparent in the cross-sectional comparison at baseline and in the change over an average of 3.2 years of follow-up. Greater decline in memory in those with apolipoprotein E4 occurred irrespective of baseline levels of global cognitive function (as assessed by MMSE). This suggested that the phenotype has a general population effect, not just an influence in those with signs of impaired cognition. Furthermore, the association between apolipoprotein E4 and greater decline on the IADL index indicates that, at a population level, this phenotype has clinically important effects even in those who do not exhibit frank dementia. In contrast to the changes in memory performance, over this time scale, this genetic variation affected tests of the attention and processing cognitive domain to a much lesser extent. Plasma lipoprotein levels had limited influence on cognition at baseline and on its decline during follow-up.

After the initial reports of an association between Alzheimer’s disease and apolipoprotein E phenotype,7 it has been demonstrated repeatedly that subjects possessing apolipoprotein E4, particularly homozygotes, have a high risk of developing dementia.10–15 The underlying mechanism is thought in part to be more beta-amyloid deposits in the presence of E4 than with other isoforms of the apolipoprotein,8,43 although it is increasingly clear that apolipoprotein E phenotype has multiple effects on neurobiology.21 Alzheimer’s disease, characterized by episodic memory loss, is likely to be the end result of a long subclinical degenerative process, and the question arises as to when and how apolipoprotein E4 contributes to this less-obvious pathology. As has been noted,11,13 early studies of the influence of apolipoprotein E4 on the rate of cognitive decline in the apparently normal population involved small numbers of subjects and included those with frank dementia or cognitive impairment at baseline or during subsequent evaluations. For example, one study44 reported a greater decrease in MMSE score and in information processing speed in elderly subjects who were apolipoprotein E4 carriers; results for memory function were less clearcut, showing no significant drop. In that study, there was a fall of 0.8 to 1.4 points in the MMSE score, indicating that a number of subjects developed significant cognitive impairment during the assessment period. In the Cognitive Function and Ageing Study, apolipoprotein E phenotype was linked to risk of dementia (as determined according to MMSE score) in the cross-sectional survey, but no association was seen with incident dementia over 6 years.45 Results supporting a link between apolipoprotein E4 and memory impairment came from a study of 611
elderly clergymen, in which it was seen that $E_4$ had a more pronounced influence on the decline in episodic memory than on other cognitive domains.\textsuperscript{46} Again, the 16.7% incidence of clinically evident Alzheimer’s disease may have influenced the result. More recently, in a report on the long-term follow-up of cognitive function in subjects aged 70 to 79 who were free of obvious dementia at baseline, apolipoprotein $E_4$ was associated at 7 years but not at 3 years of follow-up. Because of short follow-up, few developed dementia or poor general cognitive function. (Mean decrease in MMSE score was 0.13, and 89.3% of $E_4^+$ and 92.8% of $E_4^-$ subjects had MMSE scores $>24$ at their final assessment.) Dementia was not a formal study-specific diagnosis but was recorded as an adverse event. On entry, subjects were selected to have a history of, or to be at risk of, a vascular event, and the results should be extrapolated to the general public in light of this design feature. In addition, half of the subjects were allocated to receive pravastatin, but the drug had no discernible effect on cognitive decline, and it is likely that treatment did not confound the result.\textsuperscript{27}

Overall, a consistent influence of apolipoprotein $E_4$ on memory function in the immediate and delayed recall tests was observed at baseline and during follow-up. This contrasted with the relative lack of influence of $E_4$ on attention and processing (assessed by the Letter-Digit Coding test at baseline and follow-up and the Stroop test at follow-up). Furthermore, there was no evidence of a gene dosage effect, with $E_4$ homozygotes faring worse than heterozygotes. These results echo the findings of a meta-analysis of cross-sectional studies by,\textsuperscript{11} which concluded that, in the general aging population, the influence of apolipoprotein $E_4$ genotype is small in magnitude and specific to certain domains of cognitive function. The findings of the current study, together with other\textsuperscript{11,15} but not all\textsuperscript{13,20} comparable observations, raise the possibility that apolipoprotein $E_4$ is a determinant of the trajectory of memory loss (and consequent cognitive decline) in the elderly general population.

### Table 4. Comparison of Baseline Measures of Cognition and Cognitive Decline According to Tertile of Low-Density Lipoprotein Cholesterol (LDL-C) and High-Density Lipoprotein Cholesterol (HDL-C)

<table>
<thead>
<tr>
<th>Cognitive Test</th>
<th>LDL-C</th>
<th></th>
<th></th>
<th></th>
<th>HDL-C</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tertile</td>
<td>Baseline Mean (SE)</td>
<td>$P$-Value</td>
<td>Change Mean (SE)</td>
<td>$P$-Value</td>
<td>Baseline Mean (SE)</td>
<td>$P$-Value</td>
<td>Change Mean (SE)</td>
</tr>
<tr>
<td>Stroop Part III (seconds to complete)</td>
<td>I</td>
<td>71.1 (0.88)</td>
<td>.07</td>
<td>$+5.08$ (0.80)</td>
<td>.21</td>
<td>69.4 (0.90)</td>
<td>.07</td>
<td>$+6.23$ (0.82)</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>68.8 (0.89)</td>
<td>$+5.09$ (0.81)</td>
<td>71.1 (0.91)</td>
<td>$+5.20$ (0.83)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>69.0 (0.91)</td>
<td>$+6.37$ (0.83)</td>
<td>68.9 (0.93)</td>
<td>$+4.92$ (0.84)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Letter-Digit Coding (number correct)</td>
<td>I</td>
<td>22.0 (0.24)</td>
<td>.01</td>
<td>$-1.71$ (0.16)</td>
<td>.33</td>
<td>22.4 (0.25)</td>
<td>.92</td>
<td>$-1.75$ (0.17)</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>22.4 (0.24)</td>
<td>$-1.75$ (0.16)</td>
<td>22.0 (0.25)</td>
<td>$-1.73$ (0.17)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>22.5 (0.25)</td>
<td>$-1.79$ (0.17)</td>
<td>22.5 (0.26)</td>
<td>$-1.76$ (0.17)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Picture-Word Recall—immediate (number recalled)</td>
<td>I</td>
<td>9.21 (0.065)</td>
<td>.25</td>
<td>$-0.44$ (0.064)</td>
<td>.53</td>
<td>9.22 (0.066)</td>
<td>.88</td>
<td>$-0.35$ (0.065)</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>30 (0.065)</td>
<td>$-0.36$ (0.064)</td>
<td>9.29 (0.068)</td>
<td>.45</td>
<td>9.29 (0.068)</td>
<td>.39</td>
<td>$-0.44$ (0.068)</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>9.27 (0.065)</td>
<td>$-0.44$ (0.066)</td>
<td>9.29 (0.068)</td>
<td>.45</td>
<td>9.29 (0.068)</td>
<td>.39</td>
<td>$-0.44$ (0.068)</td>
</tr>
<tr>
<td>Picture-Word Recall—delayed (number recalled)</td>
<td>I</td>
<td>9.93 (0.092)</td>
<td>1.00</td>
<td>$-0.74$ (0.092)</td>
<td>.75</td>
<td>9.90 (0.094)</td>
<td>.62</td>
<td>$-0.62$ (0.094)</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>9.94 (0.093)</td>
<td>$-0.54$ (0.093)</td>
<td>10.01 (0.096)</td>
<td>.64</td>
<td>10.01 (0.096)</td>
<td>.64</td>
<td>$-0.64$ (0.096)</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>9.93 (0.096)</td>
<td>$-0.73$ (0.095)</td>
<td>9.93 (0.097)</td>
<td>.74</td>
<td>9.93 (0.097)</td>
<td>.74</td>
<td>$-0.74$ (0.097)</td>
</tr>
<tr>
<td>Barthel Index (score)</td>
<td>I</td>
<td>19.57 (0.026)</td>
<td>.001</td>
<td>$-0.61$ (0.065)</td>
<td>.59</td>
<td>19.59 (0.026)</td>
<td>.08</td>
<td>$-0.58$ (0.066)</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>19.65 (0.026)</td>
<td>$-0.52$ (0.061)</td>
<td>19.62 (0.026)</td>
<td>.56</td>
<td>19.62 (0.026)</td>
<td>.56</td>
<td>$-0.56$ (0.067)</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>19.67 (0.026)</td>
<td>$-0.58$ (0.067)</td>
<td>19.68 (0.027)</td>
<td>.57</td>
<td>19.68 (0.027)</td>
<td>.57</td>
<td>$-0.57$ (0.068)</td>
</tr>
<tr>
<td>Instrumental activity of daily living index (score)</td>
<td>I</td>
<td>13.35 (0.035)</td>
<td>.01</td>
<td>$-1.06$ (0.078)</td>
<td>.96</td>
<td>13.38 (0.034)</td>
<td>.06</td>
<td>$-1.08$ (0.079)</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>13.46 (0.036)</td>
<td>$-1.01$ (0.076)</td>
<td>13.41 (0.037)</td>
<td>.97</td>
<td>13.41 (0.037)</td>
<td>.97</td>
<td>$-1.06$ (0.081)</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>13.45 (0.036)</td>
<td>$-1.06$ (0.080)</td>
<td>13.46 (0.037)</td>
<td>.96</td>
<td>13.46 (0.037)</td>
<td>.96</td>
<td>$-1.06$ (0.082)</td>
</tr>
<tr>
<td>Mini-Mental State</td>
<td>I</td>
<td>27.9 (0.054)</td>
<td>.51</td>
<td>$-0.30$ (0.068)</td>
<td>.61</td>
<td>27.9 (0.055)</td>
<td>.72</td>
<td>$-0.28$ (0.070)</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>27.9 (0.054)</td>
<td>$-0.25$ (0.068)</td>
<td>27.9 (0.056)</td>
<td>.28</td>
<td>27.9 (0.056)</td>
<td>.28</td>
<td>$-0.28$ (0.071)</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>27.9 (0.055)</td>
<td>$-0.30$ (0.071)</td>
<td>27.9 (0.056)</td>
<td>.29</td>
<td>27.9 (0.056)</td>
<td>.29</td>
<td>$-0.29$ (0.072)</td>
</tr>
</tbody>
</table>
Vascular dementia and Alzheimer’s disease have a considerable degree of overlap in terms of neuropsychological impairments, although there is a tendency for vascular dementia to be associated with greater deficits in attention, speed of information processing, and executive function, with low HDL-C being linked to poorer cognitive function. Analogous findings were reported in the Leiden 85 Plus study, with low HDL-C being linked to poorer cognitive function. The findings of the current study is that less-able elderly people are nutritionally compromised, and this leads to both cognitive and functional deficits. The apolipoprotein E4 isoform was linked also to more-pronounced deterioration in indices of ADLs and IADLs.

In conclusion, this large-scale cognition study embedded in a clinical trial revealed that, over an average 3.2-year period, the presence of the apolipoprotein E4 isoform was associated with greater decline in memory performance without apparently affecting attention or processing ability. The apolipoprotein E4 isoform was linked also to more-pronounced deterioration in indices of ADLs and IADLs.

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Data and Safety Monitoring Committee: W. V. Brown (Chairman), H. C. Diener, J. Feely, I. Ford (Nonvoting), T. Pearson, S. Pocock, P. A. van Zwieten.

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Sponsor’s Role: PROSPER was sponsored by Bristol-Myers Squibb Ltd and company personnel on the Executive Committee helped design and conduct the trial. The sponsor had no role in the present analysis.

REFERENCES


