The apolipoprotein E epsilon2 allele and decline in episodic memory

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Objective: The apolipoprotein E (apoE) epsilon4 allele is related to decline in multiple cognitive domains, especially episodic memory, but the effect of the epsilon2 allele on change in different forms of cognitive function has been difficult to establish.

Methods: Participants are from the Religious Orders Study. At baseline, they were at least 65 years old and free of clinical evidence of dementia. For up to eight years, they underwent annual clinical evaluations that included detailed cognitive function assessment from which previously established summary measures of episodic memory, semantic memory, working memory, perceptual speed, and visuospatial ability were derived. Growth curve models were used to assess change in each measure and its relation to apoE genotype, controlling for age, sex, education, and baseline level of cognition. Follow-up data were available in 669 persons (98% of those eligible). We treated those with the epsilon3/3 genotype as the reference group (n=425), which was contrasted with epsilon2/2, epsilon2/3; n=86), and epsilon4 (epsilon3/4, epsilon4/4; n=158) subgroups.

Results: Rate of episodic memory change in the three subgroups significantly differed, with an average annual increase of 0.016 units in the epsilon2 subgroup and annual decreases of 0.022 units in those with epsilon3/3 and of 0.073 units in the epsilon4 subgroup. The epsilon2 subgroup did not differ from those with epsilon3/3 in rate of decline in other cognitive systems. The epsilon4 subgroup declined more rapidly than those with epsilon3/3 in semantic memory and perceptual speed but not in working memory or visuospatial ability.

Conclusion: Possession of one or more apoE epsilon2 alleles is associated with reduced decline in episodic memory in older persons.

Alzheimer’s disease (AD) is the most common cause of dementia in older persons. Although a small proportion of disease can be explained by rare mutations on one of three chromosomes, most AD is thought to result from a complex interaction between environmental and genetic risk factors. One well-established risk factor for AD is apolipoprotein E (apoE) status. The apoE gene has three important alleles (epsilon2, epsilon3, epsilon4), which yield six genotypes (epsilon2/2, epsilon2/3, epsilon2/4, epsilon3/3, epsilon3/4, epsilon4/4). Possession of one or more copies of the epsilon4 allele is associated with an increased risk of AD.4 The epsilon4 allele is also associated with more rapid cognitive decline in older persons,4 especially in episodic memory.4 Because impaired episodic memory is an early and defining feature of AD, these findings suggest that epsilon4 affects risk of AD mainly by augmenting the usual biological process that leads to disease.

Knowledge about the comparatively rarer epsilon2 allele has been slower to accumulate. Possession of the epsilon2 allele has been associated with a reduced risk of AD in some studies,5,6 but it has been hard to establish whether epsilon2 protects against cognitive decline and if so, whether this effect, like that of epsilon4, is especially pronounced in episodic memory. Few longitudinal cognitive function studies have focused on the epsilon2 allele,7,10-13 and results have been varied.

We used data from the Religious Orders Study, a longitudinal clinical-pathological study of aging and AD, to examine the association of the apoE epsilon2 allele with change in different cognitive systems. For up to eight years, older Catholic clergy members underwent annual clinical evaluations, including detailed cognitive function testing from which previously established composite measures of episodic memory and other cognitive functions were derived. We assessed epsilon2 effects, we contrasted an epsilon2 subgroup (consisting of epsilon2/2 and epsilon2/3) with an epsilon3/3 reference group. We assessed epsilon4 effects in a similar manner, by contrasting an epsilon4 subgroup (epsilon3/4, epsilon4/4) with epsilon3/3 to provide another point of comparison for epsilon4, and because most previous research on epsilon4, including an earlier study of this cohort,7 has grouped epsilon2/2, epsilon2/3, epsilon3/3 into a single “no epsilon4” comparison group, with the result that few published estimates of epsilon4 effects on cognitive decline are independent of epsilon2 effects.10

METHODS

Subjects
Participants are from the Religious Orders Study, a clinical-pathological investigation of aging and AD in older Catholic clergy members. They were recruited from about 40 groups across the USA (see acknowledgements) and agreed to annual clinical evaluations and brain donation at death. The study was approved by the Institutional Review Board of Rush-Presbyterian-St Luke’s Medical Center.

Clinical evaluations began in January of 1994 and new participants continue to be enrolled. Of 908 persons who had completed the baseline evaluation at the time of these analyses, apoE genotype was unavailable in 111, and 72 met dementia criteria (see below). Because we wanted to assess the independent effects associated with the epsilon2 and epsilon4 alleles, we also excluded those with the epsilon2/4 genotype (n=16). This left 709 persons eligible at baseline, 25 of whom died before their first follow-up evaluation, leaving 684 persons who were eligible for follow up. Of these, 669 persons (98%) completed at least one follow-up evaluation (mean of 6.0 evaluations per person, range: 2 to 9). Analyses are based on this group.

Clinical evaluation
At baseline, each participant underwent a uniform clinical evaluation that included a medical history, neurological...
Table 1  Descriptive information about participants in the apoE subgroups at baseline

<table>
<thead>
<tr>
<th>Variable</th>
<th>ApoE subgroup†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ε2</td>
</tr>
<tr>
<td>Number of persons</td>
<td>86</td>
</tr>
<tr>
<td>Mean (SD) age (y)</td>
<td>75.7(7.3)</td>
</tr>
<tr>
<td>Mean (SD) education (y)</td>
<td>17.7(2.8)</td>
</tr>
<tr>
<td>Women (%)</td>
<td>67.4</td>
</tr>
<tr>
<td>White, non-Hispanic (%)</td>
<td>93.0</td>
</tr>
<tr>
<td>Mean (SD) MMSE</td>
<td>28.3(1.8)</td>
</tr>
</tbody>
</table>

†The ε2 subgroup included ε2/2 and ε2/3 genotypes, ε3 included ε3/3, and ε4 included ε3/4 and ε4/4.

Table 2  Summary of random effects model examining the association of time, apoE subgroup, and their interaction with episodic memory function.

<table>
<thead>
<tr>
<th>Model term†</th>
<th>Estimate</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>−0.022*</td>
<td>0.009</td>
</tr>
<tr>
<td>ε2</td>
<td>0.089</td>
<td>0.061</td>
</tr>
<tr>
<td>ε2 × time</td>
<td>0.038*</td>
<td>0.019</td>
</tr>
<tr>
<td>ε4</td>
<td>−0.061</td>
<td>0.049</td>
</tr>
<tr>
<td>ε4 × time</td>
<td>−0.051***</td>
<td>0.015</td>
</tr>
</tbody>
</table>

†Those with the ε3/3 genotype were the reference group for contrasts with the ε2 (ε2/2, ε2/3) and ε4 (ε3/4, ε4/4) subgroups.
* p<0.05; ** p<0.01

Apolipoprotein E genotyping
Blood was collected at each site with acid citrate dextrose anticoagulant and stored at room temperature until undergoing lymphocyte separation within 24 hours of collection. DNA was extracted from about two to three million cells. Genotyping was performed by an investigator blinded to all clinical and postmortem data following the method of Hixon and Vernier.

Data analysis
Participants were divided into three apoE subgroups for all analyses: ε2, consisting of the ε2/2 and ε2/3 genotypes; ε3, consisting of ε3/3; and ε4, consisting of ε3/4 and ε4/4. Because we wanted to assess the independent contributions of ε2 and ε4 to cognition, those with the ε2/4 genotype were excluded from all analyses except the computation of allele frequencies at baseline.

We used a proportional hazards model to assess the relative risk of developing AD in the ε2 and ε4 subgroups compared with the ε3 reference group, controlling for the potentially confounding effects of age, sex, and education.

We used random effects regression models to characterise individual paths of change in each cognitive measure and to test the association of apoE genotype with initial level of function and rate of change. In this approach, variation is partitioned into that coming from persons following different paths and that coming from the observed measurements deviating from these paths. Each person’s path was assumed to follow the path of the group except for random effects that caused a given person’s baseline level of function (random intercept) to be at a higher or lower level and the rate of change (random slope) to be faster or slower. These two components of between person variability were used to estimate individual growth curves which were plotted.

Those with the ε3/3 genotype served as the reference group in all analyses. Each model included terms for time since baseline (in years), apoE subgroups ε2 and ε4 (each contrasted with the ε3 reference group), and the interaction of each subgroup with time. The term for time indicates the average annual rate of change in the ε3/3 reference group. The
terms for apoE subgroup (e2 or e4) indicates the average difference at baseline between each apoE subgroup and the reference group. The interaction terms denote the average difference in annual rate of change between each apoE subgroup and the reference group. Because of the association of cognitive function with demographic variables, all models also included terms for age, sex, education, and their interactions with time.

Model assumptions of linearity, normality, and independence and homoscedasticity of errors were evaluated graphically and analytically and were found to be adequately met. All analyses were carried out in SAS.

RESULTS

ApoE subgroups

The allele frequencies in the cohort at baseline, 0.077 for e2, 0.788 for e3, and 0.136 for e4, are comparable to those observed in population-based studies. Because we wanted to assess the independent contributions of the e2 and e4 alleles to cognitive function, we excluded persons with the e2/e4 genotype (n=16) and formed three subgroups: e2 (e2/e2=1; e2/e3=85), e3 (e3/e3=425), and e4 (e3/e4=149, e4/e4=9). The distributions of demographic variables and of baseline MMSE scores were similar in the three subgroups (table 1). In each subgroup, more than 95% of those eligible participated in follow up, with an average of 5.9 to 6.0 completed evaluations per person, which represents more than 95% of possible evaluations in survivors.

Change in episodic memory in apoE subgroups

We began analyses with episodic memory because of its strong association with apoE e4. At baseline, the summary measure of episodic memory ranged from −2.851 to 1.555 (mean=0.117; SD=0.616), with higher scores indicating better memory function. We constructed a random effects model to test whether the apoE subgroups differed in rate of change in episodic memory, controlling for baseline level of memory and for the potentially confounding effects of age, sex, and education (table 2).

Persons with the e3/e3 genotype declined an average of 0.022 units per year (95% CI −0.004 to −0.040), as shown by the term for time. Episodic memory in the e2 subgroup was similar to the e3/e3 reference group, as shown by the term for e2. By contrast, annual episodic memory change in the e4 subgroup was 0.038 units less than the reference group (p<0.05). Thus, on average, episodic memory performance in the e2 subgroup increased by 0.016 units per year. Episodic memory in the e4 subgroup did not differ from the reference...
group at baseline, but it declined by an additional 0.051 units per year (p<0.001).

To visually examine these effects, we plotted the paths of change in episodic memory during the eight years of observation in each apoE subgroup as estimated from the model (fig 1, upper left). In comparison with the e3/3 reference group, the beneficial effect of e2 and the deleterious effect of e4 on change in episodic memory are of comparable size.

To examine individual differences within the apoE subgroups, we estimated from the model the person specific paths of change in episodic memory during the eight years of observation for every person randomly selected from the e3 and e4 groups (fig 2). The horizontal axis shows the person’s age at each evaluation, and the length of each line relative to the horizontal axis shows the years of observation on that person. Heterogeneity is evident in each subgroup, but the relative absence of decline in the e2 subgroup is striking.

**Change in other cognitive domains in apoE subgroups**
We repeated the initial analysis on the summary measures of semantic memory, working memory, perceptual speed, and visuospatial ability (table 3, fig 1). On average, those with the e3/3 genotype declined in each cognitive domain. The e2 subgroup did not significantly differ from the e3/3 reference group in baseline level of function or rate of change in any of the cognitive domains, though there was a trend for reduced decline in working memory (p=0.096). The e4 subgroup did not differ from the reference group at baseline and declined more rapidly in semantic memory and perceptual speed but not in working memory or visuospatial ability.

**Incident AD in apoE subgroups**
During follow up, 124 persons developed AD, 13 (15%) in the e2 subgroup, 72 (17%) in the e3 subgroup, and 39 (25%) in the e4 subgroup. The relative risk of incident AD was 0.76 (95% CI 0.40 to 1.44) in the e2 subgroup and 1.86 (95% CI 1.22 to 2.82) in the e4 subgroup, as estimated in a proportional hazards model adjusted for age, sex, and education.

**DISCUSSION**
In a large cohort of older persons examined annually for an average of five years, possession of one or more copies of the apoE e2 allele was associated with rate of change in episodic memory but not with change in other cognitive systems. Episodic memory performance improved slightly in those with at least one e2 allele. By contrast, episodic memory declined slightly in those with the e3/3 genotype and more sharply in those with at least one e4 allele. The results suggest that the apoE e2 allele protects against episodic memory decline in older persons.
Tables 3 Summary of random effects models examining the association of time, apoE subgroup, and their interaction with function in different cognitive domains. Terms for age, sex, education, and their interactions with time were included in each model.

<table>
<thead>
<tr>
<th>Cognitive measure</th>
<th>Model term</th>
<th>Estimate</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semantic memory</td>
<td>Time</td>
<td>-0.042***</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>e2</td>
<td>-0.089</td>
<td>0.065</td>
</tr>
<tr>
<td></td>
<td>e2 x time</td>
<td>0.017</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>e4</td>
<td>0.005</td>
<td>0.051</td>
</tr>
<tr>
<td></td>
<td>e4 x time</td>
<td>-0.034*</td>
<td>0.014</td>
</tr>
<tr>
<td>Working memory</td>
<td>Time</td>
<td>-0.030***</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>e2</td>
<td>-0.060</td>
<td>0.069</td>
</tr>
<tr>
<td></td>
<td>e2 x time</td>
<td>0.020</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>e4</td>
<td>-0.019</td>
<td>0.054</td>
</tr>
<tr>
<td></td>
<td>e4 x time</td>
<td>-0.011</td>
<td>0.009</td>
</tr>
<tr>
<td>Perceptual speed</td>
<td>Time</td>
<td>-0.077***</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>e2</td>
<td>-0.046</td>
<td>0.086</td>
</tr>
<tr>
<td></td>
<td>e2 x time</td>
<td>0.022</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td>e4</td>
<td>-0.057</td>
<td>0.068</td>
</tr>
<tr>
<td></td>
<td>e4 x time</td>
<td>-0.030*</td>
<td>0.014</td>
</tr>
<tr>
<td>Visuospatial ability</td>
<td>Time</td>
<td>-0.014*</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>e2</td>
<td>0.091</td>
<td>0.075</td>
</tr>
<tr>
<td></td>
<td>e2 x time</td>
<td>0.011</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>e4</td>
<td>-0.080</td>
<td>0.059</td>
</tr>
<tr>
<td></td>
<td>e4 x time</td>
<td>-0.017</td>
<td>0.011</td>
</tr>
</tbody>
</table>

†Those with the e3/3 genotype were the reference group for contrasts with the e2 (e2/2, e2/3) and e4 (e3/4, e4/4) subgroups. *p<0.05, ***p<0.001.

As noted above, previous research on the relation of the e2 allele to change in cognitive function has yielded mixed results. In the only previous study to assess multiple cognitive domains, those with the e2/3 genotype had reduced decline on two of five episodic memory measures and on one of five measures of other cognitive functions compared with those with e3/3, but analyses were not adjusted for the potentially confounding effects of demographic variables, and no e4 effects were observed. In other studies, e2 was associated with reduced episodic memory decline (but other cognitive functions were not assessed) and with reduced decline on one of two perceptual speed measures. By contrast, e2 was unrelated to change in cognitive function, including measures of episodic memory, in two other studies.

These inconsistent results probably reflect several factors. Firstly, the e2 allele is comparatively rare, with a frequency of about 0.08 in American and European white populations, limiting statistical power. Secondly, because cognition changes gradually in older persons and is measured with error, the ability to reliably assess change in individuals depends on the length of the study period, the number of observations per person within that period, and the use of psychometrically sound outcomes. Yet some previous studies were based on three years or less of observation, and all were based on two observations per person and used individual tests as outcomes, increasing the possibility of floor and ceiling artefacts. Another issue is the variable composition of subgroups formed to assess e2 effects. Some studies, like the present one, have excluded e2/4 from the e2 subgroup, but other studies have included it for some or all analyses. Because meta-analyses suggest that the e2/4 genotype is associated with increased risk of AD, its inclusion in an e2 subgroup may tend to obscure a beneficial effect of e2 on cognition. In addition, the e2 comparison group in this and some previous studies has been restricted to those with the e3/3 genotype, but other studies have included all persons without an e2 allele, thereby confounding e2 and e4 effects.

Progressive loss of episodic memory is a defining feature of AD. That e2, like e4, seems to have a comparatively selective effect on episodic memory consistent with the idea that apoE genotype affects risk of AD mainly by augmenting or retarding the usual biological process leading to disease rather than through some other mechanism. Clinical-pathological studies will be needed to investigate these issues.

Few previous longitudinal studies have assessed the independent contributions of the e2 and e4 alleles to change in cognitive function. We found that e2 effects on cognitive decline were about equal to those of e4, or slightly smaller, but in the opposite direction. This finding underscores the limitation of binary apoE measures that contrast people with and without a given allele and suggests that ordinal approaches to scaling the overall impact of apoE may be feasible.

The risk of developing AD was increased in those with e4. AD incidence was reduced among those with e2 but not significantly so, perhaps because of limited statistical power and the lack of an e2 effect on forms of cognition other than episodic memory.

This study has several strengths. In each apoE subgroup there was an average of about six annual evaluations per person with more than 95% follow up participation in survivors, and previously established, composite measures of specific cognitive systems were used as outcomes, increasing our ability to reliably characterise individual patterns of change in cognitive function and their relation to apoE genotype. The principal limitation is that the cohort is selected and differs in important ways from the US population. It will be important, therefore, to assess e2 effects on cognitive function in more representative groups. Also, we had only one participant with the e2/2 genotype, precluding a comparison of e2 homozygotes and heterozygotes.

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REFERENCES