Effects of the APOE ε2 Allele on Mortality and Cognitive Function in the Oldest Old

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Some studies indicate that the APOE ε2 allele may have a protective effect on mortality and mental health among the elderly adults. We investigated the effect of the APOE ε2 allele on cognitive function and mortality in 1651 members of the virtually extinct Danish 1905 birth cohort. We found no protective effect of the APOE ε2 allele on mortality compared with the APOE ε3 allele. The point estimates indicated an increased protection against cognitive decline over time for persons with the APOE ε2 allele. Cognitive score did not significantly modify the mortality risk of the various APOE genotypes. We did not find a protective effect of the APOE ε2 allele on mortality among the oldest old, but in agreement with our previous findings, we found a 22% increased mortality risk for APOE ε4 carriers. The APOE ε2 allele may be protective on cognitive decline among the oldest old.

Key Words: APOE—Cognitive function—Mortality—Aging—Oldest old.

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A major question in human longevity research is how genetic variation influences our life span. Twin studies have found that genetic factors account for about a quarter of the variation in human life span in contemporary Nordic populations (1,2) and that the genetic factors may have a stronger influence at the highest ages (3,4). APOE is the most well-documented gene found to influence life span. The APOE ε4 allele is associated with an increased mortality risk throughout adulthood (5), and the effect has been found to increase with age among the oldest old (3).

The APOE allele has previously been associated with increased risk of various diseases including Alzheimer’s disease (6) and cardiovascular diseases (7–9). Most studies have focused on the negative effect of carrying the APOE ε4 allele, and fewer studies have examined the effects of the three APOE alleles on mortality separately. In some studies, the presence of the APOE ε2 allele in the oldest old has been associated with a reduced risk of dementia (10), decreased cholesterol levels (11), and an overall reduced mortality (12). Other studies suggest an increased risk of specific diseases such as Parkinson disease (10,13) among APOE ε2 allele carriers, but most studies suggest a protective effect against Alzheimer’s disease (14–17).

In relation to cognitive function, the APOE ε2 allele has previously been associated with a better memory (18,19). A recent large study of a Swedish cohort of persons aged more than 75 years found that dementia accounts for most of the increased mortality risk for carriers of the APOE ε4 variant. The study also found a protective effect of the APOE ε2 allele on mortality for women when adjusting for dementia (12). Previous cross-sectional findings in the Danish 1905 cohort suggested no significant protective effect of the APOE ε2 allele on the level of cognitive function (20).

Hence, overall there is still controversy whether the APOE ε2 allele has a protective effect on mortality and mental health among the elderly adults.

Here we investigated the effect of the APOE ε2 allele on cognitive function and mortality in the Danish 1905 birth cohort from 1998 through 2010.

Methods

Study Population

The Danish 1905 Cohort Survey is a nationwide longitudinal survey consisting of all individuals born in Denmark in 1905 identified through the Danish Civil Registration System. At baseline in 1998, a total of 3,600 persons aged 92–93 years were still alive in the cohort, and 2,262 of these persons participated in the intake survey (63%). DNA is available for 1,651 of the participants.
The baseline interview in 1998 and successive follow-ups (2000, 2003, and 2005) consisted of a personal interview in the respondent’s home and included information on sociodemographic variables, health measures, and self-reported activities of daily living. Trained interviewers from the Danish National Institute of Social Research carried out the survey. A nonresponse analysis showed no difference between responders and nonresponders with respect to sex (women, 84% vs. 89%, p = .16), median number of hospitalizations (p = .56), or median number of bed days (p = .71) during the years 2003 and 2004 (21).

DNA Analysis
Blood samples were taken from persons who were able to give informed consent. DNA was extracted using QIAamp DNA Mini kit (Qiagen, Hilden, Germany), and predesigned TaqMan SNP genotyping assays (Applied Biosystems, Foster City, CA) were used to genotype the two polymorphisms, rs429358 and rs7412, allowing for grouping into the APOE genotypes.

Interview Content
The participants were invited to a home-based 2-hour multidimensional interview, covering a wide range of questions and tests (22). The cognitive function was measured using a cognitive composite score (23,24). The specific tasks included a fluency task, which involved the number of animals an individual could name in a 1-minute interval, forward and backward digit span, and immediate and delayed recall of a 12-item list. A cognitive composite score was found by summing the five components. A higher cognitive composite score indicates a better cognitive function (23,24). Cognitive score was further dichotomized into “high” cognitive score (cognitive score above 0) and “low” cognitive score (cognitive score at 0 or less). Analyses of dichotomized cognitive function and its components by APOE haplotypes at each study wave were done using logistic regression analysis and odds ratios, and 95% confidence intervals were calculated.

Statistical Analysis
Information on date of death in the study populations up to the January 1, 2011 was retrieved from the Central Population Registry, which holds information about the total Danish population since 1968 (25) and was linked to the data using a unique identification number given to all Danish citizens. Therefore, a complete and accurate follow-up was done for all participants. In the initial descriptive analysis, we calculated age-specific, cumulated mortality rates and stratified by sex and cognitive score. Mortality rates were analyzed as a function of covariates using multiplicative Poisson regression models (26), and mortality rate ratios and 95% confidence intervals were thereby estimated. Cognitive function could be considered intermediate factors between APOE genotype and mortality rather than a possible confounder, and separate Poisson regression models were done for each stratum of cognitive score. When analyzing mortality, we used the most recently measured (eg, the last study wave where the person participated) to address cognitive score allowing participants to change group during follow-up, thus making it possible to include cognitive score as a time-dependent variable. All statistical analyses were done using SAS (version 9.2; SAS Institute Inc.).

RESULTS
At baseline, 19% and 22% of the participants were carrying the APOE ε2 allele and APOE ε4 allele, respectively (Table 1). There was a similar distribution on the different APOE subgroups by sex (Table 1). No significant differences in mean cognitive score and its components among APOE subgroups were present at baseline or over study waves. When dichotomized into high or low cognitive score, the distribution on each APOE subgroup followed that of the general distribution on each genotype (Table 1).

The overall cumulative mortality rates of APOE ε2 allele carriers were similar to the cumulative mortality rates for persons with the APOE ε33 diplotype (Figure 1A). APOE ε4 allele carriers had the highest cumulative mortality rates. When stratifying by sex, men had the highest cumulative mortality rates (Figure 1B). For men, the cumulative mortality rates of APOE ε2 allele carriers and the APOE ε33 diplotype were similar, whereas APOE ε4 allele carriers had higher cumulative mortality rates until the age of around 100 years. For women, there was a slightly lower mortality for APOE ε2 allele carriers compared with the APOE ε33 diplotype, and APOE ε4 allele carriers had the highest cumulative mortality rates. When stratified by high or low cognitive score, APOE ε4 allele carriers had the highest cumulative mortality rates, and the APOE ε2 allele carriers and the APOE ε33 diplotype had approximately the same cumulative mortality rates (Figure 1C).

The relative risk of dying for the APOE ε2 allele carriers was not significantly different from that of the APOE ε33 diplotype. This was true for both the unadjusted estimate (RR: 0.94, 95% CI: 0.82–1.08) and when adjusting for sex and age (RR: 0.96, 95% CI: 0.83–1.10) although there were tendencies that APOE ε2 allele carriers had a lower risk of dying. Carriers of the APOE ε4 allele had an unadjusted (RR: 1.20, 95% CI: 1.05–1.36) and an adjusted (RR: 1.22, 95% CI: 1.07–1.39) significantly higher risk of dying when compared with the APOE ε33 diplotype. When stratified by cognitive score, no significant protective effect on mortality was found for the APOE ε2 allele carriers when compared with the participants with the APOE ε33 diplotype (Figure 2). Carriers of the APOE ε4 allele had a significantly higher risk of dying when compared with the APOE ε33 diplotype for both groups of cognitive score (Figure 2). When further stratified by sex, no major changes in risk pattern were found (Figure 2).
The age and sex adjusted odds ratios for having a low cognitive score at study waves indicated a protective effect of carrying the APOE ε2 allele compared with the APOE ε33 diplotype, and the effect seemed to increase with study wave (Figure 3). In 2003, the difference between the APOE ε2 allele compared with the APOE ε33 diplotype reached significance with an odds ratio of 0.58 (95% CI: 0.34–0.98). The relative risk estimates for having a low cognitive function for APOE ε4 allele carriers seemed to increase with study wave when compared with the APOE ε33 diplotype (Figure 3A). This pattern was generally present for the dichotomized subcomponents of the cognitive score even though none of these reached statistical significance (Figures 3B–3F).

**Table 1. Characteristics of the Different APOE Subgroups**

<table>
<thead>
<tr>
<th>Variable</th>
<th>APOE ε22 + ε23N (%)</th>
<th>APOE ε34 + ε44N (%)</th>
<th>APOE ε33N (%)</th>
<th>APOE ε24N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>78 (17)</td>
<td>87 (19)</td>
<td>280 (61)</td>
<td>11 (3)</td>
</tr>
<tr>
<td>Women</td>
<td>185 (16)</td>
<td>214 (18)</td>
<td>722 (63)</td>
<td>39 (3)</td>
</tr>
<tr>
<td>Year</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1998</td>
<td>263 (16)</td>
<td>301 (19)</td>
<td>1002 (62)</td>
<td>50 (3)</td>
</tr>
<tr>
<td>2000</td>
<td>150 (17)</td>
<td>150 (17)</td>
<td>550 (62)</td>
<td>34 (4)</td>
</tr>
<tr>
<td>2003</td>
<td>70 (18)</td>
<td>57 (16)</td>
<td>241 (63)</td>
<td>13 (3)</td>
</tr>
<tr>
<td>2005</td>
<td>38 (21)</td>
<td>26 (15)</td>
<td>113 (63)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Fluency task (mean, standard deviation)*</td>
<td>(12.7, 6.5)</td>
<td>(12.4, 6.7)</td>
<td>(12.7, 6.8)</td>
<td>(11.6, 6.3)</td>
</tr>
<tr>
<td>High</td>
<td>122 (16)</td>
<td>137 (18)</td>
<td>476 (63)</td>
<td>18 (3)</td>
</tr>
<tr>
<td>Low</td>
<td>141 (16)</td>
<td>164 (19)</td>
<td>526 (61)</td>
<td>32 (4)</td>
</tr>
<tr>
<td>Forward digit span (mean, standard deviation)*</td>
<td>(4.7, 2.0)</td>
<td>(4.4, 2.0)</td>
<td>(4.6, 2.0)</td>
<td>(4.3, 1.7)</td>
</tr>
<tr>
<td>High</td>
<td>136 (17)</td>
<td>139 (18)</td>
<td>494 (63)</td>
<td>22 (3)</td>
</tr>
<tr>
<td>Low</td>
<td>127 (15)</td>
<td>162 (20)</td>
<td>508 (62)</td>
<td>28 (3)</td>
</tr>
<tr>
<td>Backward digit span (mean, standard deviation)*</td>
<td>(3.1, 1.7)</td>
<td>(3.1, 1.8)</td>
<td>(3.1, 1.8)</td>
<td>(2.9, 1.7)</td>
</tr>
<tr>
<td>High</td>
<td>104 (15)</td>
<td>132 (19)</td>
<td>423 (62)</td>
<td>20 (3)</td>
</tr>
<tr>
<td>Low</td>
<td>159 (17)</td>
<td>169 (18)</td>
<td>579 (62)</td>
<td>30 (3)</td>
</tr>
<tr>
<td>Immediate recall (12 items) (mean, standard deviation)*</td>
<td>(2.4, 1.8)</td>
<td>(2.2, 1.7)</td>
<td>(2.3, 1.8)</td>
<td>(2.6, 1.8)</td>
</tr>
<tr>
<td>High</td>
<td>124 (17)</td>
<td>126 (18)</td>
<td>436 (61)</td>
<td>29 (4)</td>
</tr>
<tr>
<td>Low</td>
<td>139 (15)</td>
<td>175 (19)</td>
<td>566 (63)</td>
<td>21 (2)</td>
</tr>
<tr>
<td>Delayed recall (12 items) (mean, standard deviation)*</td>
<td>(1.0, 1.6)</td>
<td>(0.9, 1.2)</td>
<td>(1.0, 1.5)</td>
<td>(0.9, 1.4)</td>
</tr>
<tr>
<td>High</td>
<td>77 (17)</td>
<td>73 (16)</td>
<td>288 (64)</td>
<td>13 (3)</td>
</tr>
<tr>
<td>Low</td>
<td>186 (16)</td>
<td>228 (20)</td>
<td>714 (61)</td>
<td>37 (3)</td>
</tr>
<tr>
<td>Cognitive score</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>122 (16)</td>
<td>140 (18)</td>
<td>484 (63)</td>
<td>22 (3)</td>
</tr>
<tr>
<td>Low</td>
<td>141 (17)</td>
<td>161 (19)</td>
<td>518 (61)</td>
<td>28 (3)</td>
</tr>
<tr>
<td>Cognitive score (mean, standard deviation)</td>
<td>(0.2, 3.5)</td>
<td>(−0.2, 3.3)</td>
<td>(0.1, 3.6)</td>
<td>(−0.2, 3.6)</td>
</tr>
<tr>
<td>1998</td>
<td>2000</td>
<td>2003</td>
<td>2005</td>
<td></td>
</tr>
</tbody>
</table>

*Measured at baseline in 1998.

The increased risk of mortality for those carrying the APOE ε4 allele has previously been demonstrated in longitudinal studies over various age groups (3,12,20,27–29), but in some studies no effect was found (30–32). Other studies have failed to observe an association between the APOE ε4 allele carriers and mortality among the oldest old (29,33,34), whereas others again have found an increased risk (3,12). This discrepancy in findings may be the results of small-scale study populations, age differences in the study populations, and length of follow-up time or different selection criteria of the study populations.

A sex-specific effect of APOE genotype on mortality has previously been studied (12,35,36). In a study based on the Kungsholmen Project (12) including 1,094 persons aged 75 years and older, the harmful effect of the APOE ε4 allele was found to be stronger in men than in women, and the results suggested a protective effect of the APOE ε2 allele on mortality in women. In our study, we did not find a stronger effect of the APOE ε4 allele on mortality in men, and we found that the APOE ε2 allele has no significant effect on mortality compared with persons with the APOE ε33 diplotype. The present study population is much older at study start than the population studied in the Kungsholmen Project, and the differences in findings may

**Discussion**

In this study based on persons aged 93 and older, we found no significant protective effect of the APOE ε2 allele on mortality compared with the APOE ε3 allele. The carriers of the APOE ε2 allele were increasingly protected against cognitive decline over time (max at 39% at third study wave). The carriers of the APOE ε4 allele had a 22% increased risk of dying compared with the APOE ε33 diplotype. Cognitive score did not significantly modify the mortality risk of the various APOE genotypes.

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be due to selection pressure through age, thus diminishing the effects of the APOE ε2 allele at advanced ages. However, previously we found an increased effect for APOE ε4 allele carriers with age in the 1905 cohort with approximately the same follow-up (3), and if genes have a stronger influence at the highest ages (3,4), it is more likely that other factors have influenced the observed discrepancy. Furthermore, there are a limited number of men alive in the present study, which makes chance an important agent in the findings.

The protective effect found for the APOE ε2 allele on the decrease in cognitive score in this study corresponds to previous findings of a protective effect on dementia (12) and reduced decline in memory among the elderly adults (10,18,19). A recent study suggests that the APOE ε2 allele protects against brain atrophy (37) and that this may be related to a higher concentration of synaptic proteins in the cerebral cortex (38), which may play a central role in memory and cognitive function.

The strengths of our research are the long follow-up period, the high participation rate, and the measure of cognitive function over time, which made it possible to include these as time-dependent covariates in the analysis. In the present study, we did not have information on causes of death. Considering that the major cause of death in Denmark is cardiovascular diseases, this could potentially explain the observed increased mortality in the APOE ε4 allele carriers. Previous studies have found an association between carrying the APOE ε4 allele and cardiovascular diseases. This may also have been the case in the present study. Our population was followed from age 92–93 years, thus making the result relevant for the oldest old but not for younger persons. Consequently, this complicates a comparison with other studies on this topic but also warrants studies among younger persons. The age groups studied here further raise the problem of survival selection, perhaps weakening the association between APOE and mortality. However, in a previous study of the Danish 1905 cohort, we did not find any attenuation of the APOE and mortality association with age (3), on the contrary, although those findings did not exclude the possibility that the association was even stronger at younger ages than the ones studied here. Cognitive function may be considered an intermediate factor in the causal chain between APOE genotype and mortality. Still, in the present study, stratification of the analysis by cognitive function examining the separate effect of APOE genotype within strata gave similar results. For the individual APOE genotypes studied here, no significant differences in mortality risk were found for persons with low or high cognitive score.

In conclusion, in this study, we did not find an overall protective effect of the APOE ε2 allele on mortality among the oldest old, but we found a 22% increased mortality risk for APOE ε4 allele carriers. The APOE ε2 allele has a protective effect on cognitive decline among the oldest old.
Figure 2. Relative risk of dying when compared with the APOE ε33 diplotype. Vertical lines on bars are 95% confidence intervals.

Figure 3. Odds ratio for low cognitive score and its components over study waves when compared with the APOE ε33 diplotype. Vertical lines on bars are 95% confidence intervals.
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