Apolipoprotein E Genotypes: Relationship to Cognitive Functioning, Cognitive Decline, and Survival in Nonagenarians

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OBJECTIVES: To evaluate the extent to which relationships between apolipoprotein E, cognitive functioning, and survival in people aged 60 to 80 persist into advanced old age.

DESIGN: Examine the effect of apolipoprotein E genotypes on baseline cognitive functioning, cognitive decline over 5 years, and survival in a cohort of 1,551 nonagenarians.

SETTING: The Danish 1905 birth cohort.

PARTICIPANTS: One thousand five hundred fifty-one nonagenarians from the Danish 1905 birth cohort.

MEASUREMENTS: Cognitive functioning was assessed using the Mini-Mental State Examination (MMSE) and five brief cognitive tests (cognitive composite).

RESULTS: The subjects were stratified into four groups by occurrence of a protective (ε2) or a risk (ε4) apo E allele (ε22 and ε23, ε33, ε24 and ε34, ε44). At intake, the mean scores for the three genotype groups were 22.1, 21.8, 21.4, and 21.0 for MMSE and 0.10, 0.07, − 0.02, and 0.30 for the cognitive composite, respectively. Growth-curve analyses showed that, although individuals carrying at least one ε4 allele had slightly lower MMSE scores and declined slightly more rapidly over time, this effect was not statistically significant and was not apparent in scores on the cognitive composite. In subjects whose functioning was relatively well preserved (those still living and able to participate in the assessment, and whose cognitive functioning had declined less than 4 points on the MMSE), ε4 frequencies tended to decline at subsequent waves ($P = .03$, chi-square test for trend), but ε4 had no significant survival disadvantage (hazard ratio = 1.11 (95% confidence interval = 0.99–1.25; $P = .07$).

CONCLUSION: Apo E genotype has a small effect on the probability of remaining a well-functioning nonagenarian but no separately detectable effect on cognitive functioning, cognitive decline, or survival. **J Am Geriatr Soc 54:654–658, 2006.**

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The maintenance of cognitive abilities is an important basis for successful aging and is a major component of quality of life in the oldest old. Twin studies have shown that the variation in cognitive functioning can be attributed to environmental and genetic factors but that genetic factors become increasingly important with age[1,2] and account for at least 50% of the variance in cognitive functioning in the oldest old.[3] One contributor to genetic variation, which has been replicated in many settings in younger elderly, is variability in the gene coding for apolipoprotein E (apo E)[4–8]—a 299 amino acid plasma glycoprotein that plays a major role in lipoprotein metabolism as a ligand for receptors of the low-density lipoprotein receptor superfamily. Two polymorphisms in the coding region of the apo E gene result in three major isoforms of the protein: apo E2, apo E3 (the major isoform), and apo E4. The alleles coding for these isoforms are apo E ε2, ε3, and ε4, respectively.

Dementia is the most common neurodegenerative disorder affecting older people. Most cases (60–70%) are diagnosed as Alzheimer's disease (AD), whereas the rest are due mostly to vascular dementia.[6] One of the most studied susceptibility factors for AD is apo E. Increased risk of AD and cognitive impairment is associated with the apo E ε4 allele, whereas the ε2 allele is reported to be protective. It remains controversial whether apo E is related to cognitive functioning and decline in older people without AD. Several studies have shown that the ε4 allele is a risk factor for cognitive impairment and decline,[4,6,8,9] but other studies have not observed an effect of the ε4 allele on the cognitive functioning of normal older people.[10,11] Nearly all of these studies have been conducted in people aged 60 to 80, and only a few studies have been conducted in subjects aged 85 and older, and these have generally had small sample sizes.[12,13]

Apo E genotypes are also associated with increased risk of ischemic heart disease. The risk differs as a function of apo E genotype. Relative to ε33 individuals, ε32 individuals are protected, whereas ε34 and ε44 individuals are particularly susceptible to ischemic heart disease.[14]

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Several studies have demonstrated that the ε4 allele is less common in nonagenarians and centenarians than at younger ages.[12,13,15,16] Therefore, it is likely that it is associated with excess risk of death—probably due to the fact that the ε4 allele is associated with greater risk of two major causes of death in industrialized countries: ischemic heart disease and AD. Nevertheless, it seems that the greater risk of death in the ε4 allele carriers decreases with age and that the different genotypes are associated with little variation in mortality in the oldest old.[17] Nevertheless, the calculation of risk in those aged 80 to 100 is based on few individuals.

In this study, the effect of apo E genotypes on cognitive functioning, cognitive decline, and survival was examined in a cohort of 1,551 nonagenarians.

SUBJECTS AND METHODS

Subjects and Measurement of Cognitive Abilities

The participants in this study were from the Danish 1905 birth cohort ascertained in 1998 when they were aged 92 to 93 (1,639 deoxyribonucleic acid (DNA) samples from 462 men and 1,177 women).[18] The survivors were reassessed in 2000 and 2003. The participants were invited to participate in a home-based 2-hour multidimensional interview, as previously described.[18] The interview included the Mini-Mental State Examination (MMSE)[19] and five brief individual tests of cognitive functioning selected to be sensitive to age-related memory and verbal fluency,[3] as well as collection of DNA. The MMSE is a widely used screen for cognitive impairment and yields a score between 0 and 30. Cognitive impairment is graded as severe for scores between 0 and 17, mild for scores between 18 and 23, and normal for scores between 24 and 30. A cognitive composite score was computed by aggregating performance on a fluency task (numbers of animals the individual could name within 1 minute), forward and backward digit span, and immediate and delayed recall of a 12-item list. To facilitate interpretation of results, each of the five brief individual tests of cognitive functioning was standardized to a mean of 0 and a standard deviation of 1 in the total sample before summing to form a cognitive composite score.[3] To further facilitate the interpretation of the composite score, it was rescaled to have a mean of 0 and a standard deviation of 1 for all participants who completed this assessment at initial testing. Because the scaling for the MMSE is well known, a similar transformation of this variable was not performed.

Cognitive functioning was assessed, and DNA samples were taken only from subjects who were able to perform the interview (DNA was not taken from proxy respondents). The DNA sample could be given as a blood sample or a cheek swab.

Determination of Apo E Genotype

DNA was isolated from cheek swabs and blood sample, with the use of QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The Taqman technology was used to genotype the two polymorphisms at amino acid residue 112 and 158. Primers and probes were designed using Primer Express software (Applied Biosystems, Foster City, CA). Oligonucleotide primer and probe sequences are available upon

Table 1. Neuropsychological Test Scores as a Function of Apolipoprotein E (APOE) Genotype in the Total Population and the Subgroup with Mini-Mental State Examination (MMSE) Score of 24 or More

		All Participants					MMSE ≥24				
		ε22 & ε23	ε33	ε24 & ε34	ε44		ε22 & ε23	ε33	ε24 & ε34	ε44	
Test	N	Mean ± SD				N	Mean ± SD				
MMSE*		(n = 252)	(n = 964)	(n = 318)	(n = 17)		(n = 124)	(n = 473)	(n = 130)	(n = 8)	
Men	434	22.7 ± 5.5	22.3 ± 6.0	22.2 ± 5.3	20.8 ± 11.3	235	26.8 ± 1.8	26.6 ± 1.8	26.0 ± 1.6	26.3 ± 1.5	
Women	1,117	21.9 ± 5.4	21.6 ± 5.9	21.1 ± 5.5	21.1 ± 5.6	500	26.3 ± 1.9	26.3 ± 1.8	26.2 ± 1.8	27.0 ± 1.6	
All	1,551	22.1 ± 5.5	21.8 ± 5.9	21.4 ± 5.5	21.0 ± 6.9	735	26.5 ± 1.9	26.4 ± 1.8	26.1 ± 1.7	26.8 ± 1.5	
Cognitive composite†		(n = 251)	(n = 960)	(n = 317)	(n = 17)		(n = 124)	(n = 472)	(n = 129)	(n = 8)	
Men	432	0.16 ± 1.06	0.12 ± 0.97	− 0.08 ± 1.00	0.28 ± 1.66	234	0.76 ± 0.91	0.57 ± 0.86	0.38 ± 0.81	0.95 ± 1.18	
Women	1,113	0.08 ± 0.94	0.05 ± 1.01	− 0.01 ± 0.89	0.31 ± 0.91	499	0.60 ± 0.83	0.62 ± 0.83	0.51 ± 0.80	0.75 ± 0.74	
All	1,545	0.10 ± 0.97	0.07 ± 1.00	− 0.02 ± 0.92	0.30 ± 1.07	733	0.65 ± 0.85	0.61 ± 0.84	0.47 ± 0.81	0.82 ± 0.85	

* Eighty-eight subjects (28 men and 60 women) did not complete the MMSE or did not have the APOE genotype.
† Ninety-four subjects (30 men and 64 women) did not complete the composite score or did not have the APOE genotype.

request. Twenty-three subjects could not be genotyped because of poor quality of DNA.

Statistical Analysis

The effect of apo E genotype on initial cognitive functioning and change in cognitive functioning was investigated using growth curve methods.[20] In these analyses, cognitive performance (the composite or MMSE) was modeled as a function of apo E genotype and wave of assessment. To maximize power, apo E genotype was dichotomized as presence versus absence of an ε4 allele. Growth curve analysis was completed using PROC MIXED from the SAS (SAS Institute, Inc., Cary, NC).

For the survival analyses, participants were followed from the date of blood sampling until emigration, death, or end of study period (January 2005). Information on emigration and death was retrieved from the Danish Central Population Register, which is continuously updated. The Kaplan-Meier method was used to plot cumulative survival curves. For the survival analysis, the subjects were stratified by the occurrence of the apo E ε4 allele: an apo E ε4-negative group, a group with one ε4 allele (ε24 and ε34), and a group consisting of subjects homozygous for the ε4 allele (ε44).

The proportional hazards model for survival, adjusted for sex, was used to test for a survival difference. The statistical program package Stata (Release 8.0, StataCorp., College Station, TX) was used for the statistical calculation.

RESULTS

Relation Between Apo E Genotype and Intake Cognitive Functioning

Apo E genotype and intake cognitive data (the cognitive composite or the MMSE) was available for 1,551 (average age ± standard deviation 93.1 ± 0.3) of the 1,814 individuals interviewed at intake. The mean scores for the neuropsychological tests at intake according to the different genotype groups are shown in Table 1. In total, 45.9% men and 55.2% women had a MMSE score less than 24. The scores for the isolated cognitive normal group is therefore also shown to clarify the effect of ε4 in the nondemented by deleting subjects with a possible incipient dementia as reflected by an intake MMSE score less than 24. In both situations—all participants and participants with a MMSE score of 24 or higher—the mean decreased as a function of genotype when the subjects were stratified into four groups by occurrence of a protective (ε2) or a risk (ε4) apo E allele (ε22 and ε23, ε33, ε24 and ε34, εε44). Although the findings for the MMSE and the cognitive composite were nonsignificant as revealed by the growth analyses, the means followed the expected pattern—a trend toward lower cognitive functioning in carriers of the ε4 allele.

Apo E Genotype Distribution at Intake and Follow-Up

The apo E genotype distribution at intake and the distribution in the well-functioning versus the adverse-outcome groups (death, interview by proxy, or extreme decline in MMSE at follow-up) are shown in Table 2. In total, 884 individuals (222 men, 662 women) participated at the first follow-up in 2000. Of those, 11 (3 men and 8 women) refused to participate in the cognitive measurements. At the

Table 2. Genotype Distribution at Intake (1998) and at First (2000) and Second (2003) Follow-Up

Apolipoprotein Genotype	Intake	First Follow-Up				Second Follow-Up			
		Well-Functioning	Dead	Proxy	MMSE Decline ≥4	Well-Functioning	Dead	Proxy	MMSE Decline ≥4
		n (%)							
ε22	17 (1.1)	4 (0.8)	6 (1.7)	3 (2.7)	2 (0.8)	1 (0.7)	12 (1.1)	2 (2.4)	2 (1.4)
ε23	246 (15.2)	78 (15.5)	62 (17.2)	20 (17.9)	42 (16.3)	28 (18.8)	159 (14.9)	13 (15.5)	23 (16.3)
ε33	1,002 (62.0)	331 (65.7)	218 (60.4)	65 (58.0)	146 (56.8)	97 (65.1)	650 (61.0)	53 (63.1)	87 (61.7)
ε24	50 (3.1)	15 (3.0)	3 (0.8)	6 (5.4)	13 (5.1)	2 (1.3)	32 (3.0)	5 (6.0)	5 (3.5)
ε34	281 (17.4)	71 (14.1)	70 (19.4)	17 (15.2)	51 (19.8)	19 (12.8)	198 (18.6)	11 (13.1)	22 (15.6)
ε44	20 (1.2)	5 (1.0)	2 (0.6)	1 (0.9)	3 (1.2)	2 (1.3)	14 (1.3)	0	2 (1.4)
Total	1,616	504	361	112	257	149	1,065	84	141

Note: The genotypes are listed in the well-functioning and adverse outcome groups: death, interview performed by proxy, or large decline in Mini-Mental State Examination (MMSE) score.

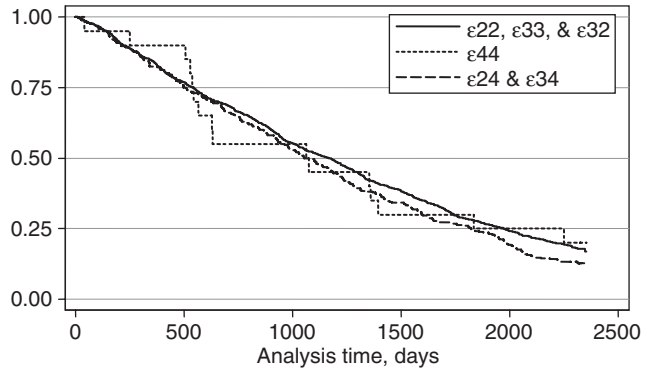

Figure 1. Kaplan-Meier survival estimates. The relationship between survival probability and occurrence of apo E ε4 alleles.

second follow-up, in 2003, 381 participated (79 men, 302 women). Of those, seven (2 men and 5 women) refused to participate in the cognitive measurements. The well functioning at first and second follow-up are defined as those still alive and able to perform the interview without the help of a proxy and with a MMSE decline (difference between MMSE at intake and MMSE at follow-up) of less than four. The mean MMSE decreased from 21.8 at intake to 21.2 at first follow-up and 20.3 at second follow-up. The frequency of the ε4 positive (ε24, ε34, and ε44) in the well-functioning groups decreased from 21.7% at intake to 18.1% at first follow-up and 15.4% at second follow-up. The chi-square test for trend testing this decrease showed a significant reduction ($P = .03$).

Growth Curve Analyses

Test statistics from the growth curve analysis of the cognitive data showed that the main effect of apo E (the effect of apo E genotype on initial cognitive performance) was not statistically significant for the cognitive composite (F statistic = 2.89; $P = .09$) or MMSE (F statistic = 1.53; $P = .22$), although the *P*-value in the former case is borderline significant. The wave-by-apo E interaction (the effect of apo E on change in cognitive performance) was also not significant for the cognitive composite (F statistic = 0.08; $P = .78$) or MMSE (F statistic = 3.27; $P = .07$), although in this case, the latter test approached statistical significance.

Relation Between Apo E Genotype and Survival

The Kaplan-Meier cumulative survival curves are shown in Figure 1. The survival difference is in the expected direction, but despite the large sample size and with 83% dead at the end of the follow-up period, there were no significant survival differences, with the proportional hazards model for survival adjusted for sex providing the hazard ratio 1.11 (95% CI = 0.99–1.25; $P = .07$); the hazard increases 11% for having an ε4 allele.

DISCUSSION

These results show that apo E genotypes are not significantly associated with cognitive functioning or cognitive decline in nonagenarians. Although the MMSE performance of individuals carrying at least one ε4 allele was worse than the MMSE performance of those carrying no ε4 alleles and declined somewhat more rapidly, this effect did not quite attain statistical significance and was moreover not observed in analysis of the cognitive composite score.

The ε4 allele has a small and nonsignificant influence on survival probability in this nonagenarian population. Several studies have examined changes in apo E genotype frequency with age and found a significant decrease in ε4 alleles as evidence of a large difference in mortality by genotype,[13,16,21] but this difference in mortality is not evident in the current study, suggesting that the apo E ε4 effects are age dependent and only minor in nonagenarians.

An association between apo E and cognitive functioning was expected, and it was surprising that this large study of 1,551 nonagenarians was too small to detect a significant difference. The lack of association is in contrast with previous studies that have established an association between AD and apo E genotypes[5,22] and a recent study that found that the variation in nonpathological cognitive changes from age 11 to 80 is related to the apo E genotype.[4] Nevertheless, the current finding that apo E ε4 only weakly predicts cognitive functioning in the oldest old supports findings from previous studies in smaller populations. One explanation for these different results could be that the effect associated with the apo E ε4 is strongest in people in their 60s and 70s and of only minor importance in the oldest old, although twin studies have shown that the heritability of cognitive abilities is substantial even for the very old.[1]

The current study shows that the apo E ε4 allele, which is a commonly known risk factor for mortality, apparently loses it importance with age. At first glance, this may appear unexpected because this allele has been strongly associated with coronary heart disease, AD, and crude mortality. The ε44 and ε34 genotypes account for almost 20% of the nonagenarians, and it should be possible to detect a major mortality difference in this large population that has a high rate of mortality (83%). Nevertheless, the high mortality in the oldest old results in a high degree of selection. The most frail and otherwise disadvantaged members will tend to die first, leaving the most robust in the population—a pattern commonly seen for risk factors[23] due to selection; those most susceptible to the risk factor are already dead. So nonagenarians with the ε4 allele apparently have a defense—genetic or environmental—that makes them less susceptible to the harmful effects connected to the ε4 allele.

To further investigate the potential role of apo E in "successful" aging, cognitive decline, and survival in these nonagenarians, the distribution of apo E genotypes in the arbitrarily defined well-functioning group was examined. There was a significant decrease in the frequency of ε4-positive subjects in the well-functioning groups from intake to first and second follow-up. So although the growth models could not reveal a significant association between cognitive functioning at intake and cognitive decline, the ε4 negative subjects are more likely to remain alive and well functioning at this high age than the ε4-positive subjects.

Identifying factors that influence cognitive abilities and survival in the oldest old have large public health implications, especially if our understanding could result in preventative and ameliorative interventions. The current study shows that the apo E genotype affects the probability of remaining a "well-functioning" nonagenarian, although

it is not possible to detect separately a significant difference in cognitive functioning, cognitive decline, or survival.

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